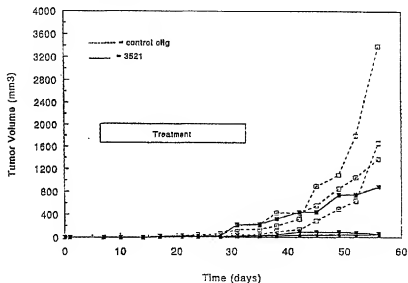




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(54) Title: OLIGONUCLEOTIDE MODULATION OF PROTEIN KINASE C



(57) Abstract

Compositions and methods are provided for the treatment and diagnosis of diseases associated with protein kinase C. Oligonucleotides are provided which are specifically hybridizable with a PKC gene or mRNA. Oligonucleotides specifically hybridizable with a particular PKC isozyme, set of isozymes or mRNA transcript are provided. Methods of treating conditions amenable to therapeutic intervention by modulating protein kinase C expression with an oligonucleotide specifically hybridizable with a PKC gene or mRNA are disclosed. Compositions and methods are provided for the treatment, detection and diagnosis of diseases associated with protein kinase Calpha and specific transcripts thereof. New nucleic acid sequences are provided which encode 3' untranslated regions of human protein kinase Calpha. Polynucleotide probes for PKCalpha are also disclosed.

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## OLIGONUCLEOTIDE MODULATION OF PROTEIN KINASE C

### FIELD OF THE INVENTION

This invention relates to therapies, diagnostics, and research reagents for disease states which respond to modulation of the expression of protein kinase C. In particular, this invention relates to antisense oligonucleotides specifically hybridizable with nucleic acids relating to protein kinase C. These oligonucleotides have been found to modulate the expression of protein kinase C. Palliation and therapeutic effect result.

### BACKGROUND OF THE INVENTION

The phosphorylation of proteins plays a key role in the transduction of extracellular signals into the cell. The enzymes, called kinases, which effect such phosphorylations are targets for the action of growth factors, hormones, and other agents involved in cellular metabolism, proliferation and differentiation. One of the major signal transduction pathways involves the enzyme protein kinase C (PKC), which is known to have a critical influence on cell proliferation and differentiation. PKC is activated by diacylglycerols (DAGs), which are metabolites released in signal transduction.

Interest in PKC was stimulated by the finding that PKC is the major, and perhaps only, cellular receptor through which a class of tumor-promoting agents called phorbol esters exert their pleiotropic effects on cells [Gescher et al., *Anti-Cancer Drug Design* 4:93-105 (1989)]. Phorbols capable of tumor production can mimic the effect

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of DAG in activating PKC, suggesting that these tumor promoters act through PKC and that activation of this enzyme is at least partially responsible for the resulting tumorigenesis [Parker et al., *Science* 233:853-866 (1986)].

- 5       Experimental evidence indicates that PKC plays a role in growth control in colon cancer. It is believed that specific bacteria in the intestinal tract convert lipids to DAG, thus activating PKC and altering cell proliferation. This may explain the correlation between
- 10   high dietary fat and colon cancer [Weinstein, *Cancer Res. (Suppl.)* 51:5080s-5085s (1991)]. It has also been demonstrated that a greater proportion of the PKC in the colonic mucosa of patients with colorectal cancer is in an activated state compared to that of patients without cancer
- 15   [Sakanoue et al., *Int. J. Cancer* 48:803-806 (1991)].

Increased tumorigenicity is also correlated with overexpression of PKC in cultured cells inoculated into nude mice. A mutant form of PKC induces highly malignant tumor cells with increased metastatic potential.

- 20       Sphingosine and related inhibitors of PKC activity have been shown to inhibit tumor cell growth and radiation-induced transformation *in vivo* [Endo et al., *Cancer Research* 51:1613-1618 (1991); Borek et al., *Proc. Natl. Acad. Sci.* 88:1953-1957 (1991)]. A number of experimental
- 25   or clinically useful anti-cancer drugs show modulatory effects on PKC. Therefore, inhibitors of PKC may be important cancer-preventive or therapeutic agents. PKC has been suggested as a plausible target for more rational design of conventional anti-cancer drugs [Gescher, A. and
- 30   Dale, I.L., *Anti-Cancer Drug Design*, 4:93-105 (1989)].

- Experiments also indicate that PKC plays an important role in the pathophysiology of hyperproliferative skin disorders such as psoriasis and skin cancer. Psoriasis is characterized by inflammation,
- 35   hyperproliferation of the epidermis and decreased differentiation of cells. Various studies indicate a role for PKC in causing these symptoms. PKC stimulation in

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cultured keratinocytes can be shown to cause hyperproliferation. Inflammation can be induced by phorbol esters and is regulated by PKC. DAG is implicated in the involvement of PKC in dermatological diseases, and is formed to an increased extent in psoriatic lesions.

Inhibitors of PKC have been shown to have both antiproliferative and antiinflammatory effects *in vitro*. Some antipsoriasis drugs, such as cyclosporine A and anthralin, have been shown to inhibit PKC. Inhibition of PKC has been suggested as a therapeutic approach to the treatment of psoriasis [Hegemann, L. and G. Mahrle, *Pharmacology of the Skin*, H. Mukhtar, ed., p. 357-368, CRC Press, Boca Raton, FL, 1992].

PKC is not a single enzyme, but a family of enzymes. At the present time at least seven isoforms (isozymes) of PKC have been identified:  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$  and  $\eta$ . These isozymes have distinct patterns of tissue and organ localization (see Nishizuka, *Nature*, 334:661-665 (1988) for review) and may serve different physiological functions. For example, PKC- $\gamma$  seems to be expressed only in the central nervous system. PKC- $\alpha$  and - $\beta$  are expressed in most tissues, but have different patterns of expression in different cell types. For example, both PKC- $\alpha$  and PKC- $\beta$  are expressed in, and have been purified from, human epidermis. While PKC- $\alpha$  has been detected mainly in keratinocytes of the basal layers of the epidermis, PKC- $\beta$  is found mainly in the middle layers of the epidermis and Langerhans cells. PKC- $\eta$  has been found predominantly in the skin and lungs, with levels of expression much higher in these tissues than in the brain. This is in contrast to other members of the PKC family which tend to be most abundantly expressed in the brain [Osada et al., *J. Biol. Chem.* 265:22434-22440 (1990)]. Another PKC isozyme, PKC- $\zeta$ , is believed to play a critical role in control of proliferative cascades. This was demonstrated by using antisense RNA, peptide inhibitors or a 15-mer phosphorothioate antisense oligonucleotide targeted to the

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AUG of *Xenopus* PKC- $\zeta$  to deplete PKC- $\zeta$  levels in *Xenopus* oocytes. These depleted oocytes were shown to be resistant to maturation in response to insulin, while the maturation pathway activated by progesterone was not affected. WO

- 5 93/20101. While the PKC isozymes listed here are preferred for targeting by the present invention, other isozymes of PKC are also comprehended by the present invention.

It is presently believed that different PKC isozymes may be involved in various disease processes depending on  
10 the organ or tissue in which they are expressed. For example, in psoriatic lesions there is an alteration in the ratio between PKC- $\alpha$  and PKC- $\beta$ , with preferential loss of PKC- $\beta$  compared to normal skin [Hegemann, L. and G. Mahrle, *Pharmacology of the Skin*, H. Mukhtar, ed., p. 357-368, CRC  
15 Press, Boca Raton, FL, 1992].

Even for a given isozyme, there may be multiple RNA transcripts expressed from a single gene. In the case of PKC $\alpha$ , for example, two mRNA transcripts are seen: a long (approximately 8.5 kb) transcript and a short  
20 (approximately 4 kb) transcript. Multiple PKC $\alpha$  transcripts are produced from the murine and the bovine PKC $\alpha$  genes as well. The ratio between the long and short transcripts varies between species and is believed to vary between tissues as well. In addition, there may be some  
25 correlation between this ratio and the proliferative state of cells.

Although numerous compounds have been identified as PKC inhibitors (see Hidaka and Hagiwara, *Trends in Pharm. Sci.* 8:162-164 (1987) for review), few have been found  
30 which inhibit PKC specifically. While the quinoline sulfonamide derivatives such as 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) inhibit PKC at micromolar concentrations, they exhibit similar enzyme inhibition kinetics for PKC and the CAMP-dependent and cGMP-dependent  
35 protein kinases. Staurosporine, an alkaloid product of *Streptomyces* sp., and its analogs, are the most potent *in vitro* inhibitors of PKC identified to date. However, they

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exhibit only limited selectivity among different protein kinases [Gescher, *Anti-Cancer Drug Design* 4:93-105 (1989)]. Certain ceramides and sphingosine derivatives have been shown to have PKC inhibitory activity and to have promise for therapeutic uses, however, there remains a long-felt need for specific inhibitors of the enzymes.

There is also a desire to inhibit specific PKC isozymes, both as a research tool and as treatment for diseases which may be associated with particular isozymes.

- 10 Godson et al. [*J. Biol. Chem.* 268:11946-11950 (1993)] recently disclosed use of stable transfection of antisense PKC- $\alpha$  cDNA in cytomegalovirus promotor-based expression vectors to specifically decrease expression of PKC- $\alpha$  protein by approximately 70%. It was demonstrated that  
15 this inhibition causes a loss of phospholipase A<sub>2</sub>-mediated arachidonic acid release in response to the phorbol ester PMA. Attempts by the same researchers at inhibiting PKC activity with oligodeoxynucleotides were ultimately unsuccessful due to degradation of oligonucleotides.

## 20 OBJECTS OF THE INVENTION

It is a principal object of the invention to provide therapies for neoplastic, hyperproliferative, inflammatory and other disease states associated with protein kinase C.

- Another object of the invention is to provide  
25 selective therapies for diseases associated with particular isozymes of protein kinase C.

It is a further object of the invention to provide antisense oligonucleotides which are capable of modulating the expression of protein kinase C.

- 30 Another object of the invention is to provide antisense oligonucleotides which are capable of selectively modulating the expression of particular isozymes of protein kinase C.

- Yet another object is to provide means for diagnosis  
35 of diseases associated with protein kinase C.

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A further object of the invention is to provide means for differential diagnosis of diseases associated with particular isozymes of protein kinase C.

- 5 provide research tools for the study of the effects of protein kinase C expression and diseases associated therewith.

- 10 An additional object of the invention is to provide research tools for the study of the effects of expression of particular isozymes of protein kinase C and diseases associated therewith.

- 15 It is an object of the invention to provide novel nucleic acid molecules encoding a 3'-untranslated region of human PKC $\alpha$ , including sequences unique to the long mRNA transcript of PKC $\alpha$ .

Another object of the invention is to provide antisense oligonucleotides which are capable of selectively modulating the expression of particular mRNA transcripts of PKC $\alpha$ .

- 20 A further object of the invention is to provide polynucleotide probes for detection of human PKC.

A still further object of the invention is to provide polynucleotide probes for detection of particular mRNA transcripts of PKC $\alpha$ .

- 25 A further object of the invention is to provide means for differential diagnosis of diseases associated with particular mRNA transcripts of PKC $\alpha$ .

- 30 It is an object of the invention to provide therapies for neoplastic, hyperproliferative, inflammatory and other disease states associated with PKC $\alpha$ .

Another object of the invention is to provide selective therapies for diseases associated with particular mRNA transcripts of PKC $\alpha$ .

- 35 An additional object of the invention is to provide research tools for the study of the effects of expression of particular transcripts of PKC $\alpha$  and diseases associated therewith.



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These and other objects of this invention will become apparent from a review of the instant specification.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1(a) and 1(b) are graphical depictions of the effects on PKC expression of antisense oligonucleotides hybridizable with PKC- $\alpha$ . Oligonucleotides are arranged by PKC target region, 5' to 3'.

Figure 2 is a line graph showing dose-dependent reduction of PKC- $\alpha$  protein levels after oligonucleotide treatment of A549 cells. ▼ = ISIS 4632; ■ = ISIS 4649; ● = ISIS 4636; ▲ = ISIS 4648.

Figure 3 is a bar graph showing reduction of PKC- $\alpha$  mRNA after treatment of A549 cells with oligonucleotides. Hatched bars represent the 8.5 kb transcript, plain bars represent the 4.0 kb transcript.

Figure 4 is a line graph showing the relationship between deoxy gap length and activity of chimeric oligonucleotides against PKC.

Figure 5 is a line graph showing dose response curves for chimeric oligonucleotides (all SEQ ID NO: 3) with different deoxy gap lengths.

Figure 6 is a bar graph showing the effects of several 2'-O-methyl chimeric oligonucleotides of SEQ ID NO: 3 on PKC- $\alpha$  mRNA levels. Hatched bars represent the 8.5 kb transcript, plain bars represent the 4.0 kb transcript.

Figure 7 is a bar graph and diagram showing the effects of several 2'-O-methyl and 2'-O-propyl chimeric oligonucleotides (6996, 7273) of SEQ ID NO: 3 on PKC- $\alpha$  mRNA levels. Hatched bars represent the 8.5 kb transcript, plain bars represent the 4.0 kb transcript.

Figure 8 is a bar graph and diagram showing the effects of additional 2'-O-methyl and 2'-O-propyl chimeric oligonucleotides (7008, 7294) of SEQ ID NO: 3 on PKC- $\alpha$  mRNA levels. Hatched bars represent the 8.5 kb transcript, plain bars represent the 4.0 kb transcript.

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Figure 9 is a set of bar graphs showing the effect of additional oligonucleotides on PKC- $\alpha$  mRNA levels. Figure 9A shows oligonucleotides 6632, 6653 and 6665. Figure 9B shows oligonucleotides 3521 (for comparison), 7082, 7083 and 7084. Hatched bars represent the 8.5 kb transcript, plain bars represent the 4.0 kb transcript.

Figure 10 is a line graph showing anti-tumor activity of ISIS 3521. Each dashed line represents tumor volume in one animal treated with control oligonucleotide; each solid line represents tumor volume in one animal treated with ISIS 3521.

Figure 11 is a set of line graphs showing effect of oligonucleotides on growth of human MDA-MB231 tumors in nude mice. Figure 11A shows results obtained with ISIS 3521; Figure 11B shows results obtained with ISIS3527. Each line represents tumor volume in one animal. • = control; o = oligonucleotide at 60 mg/kg ;  $\Delta$  = oligonucleotide at 6 mg/kg.

Figure 12 is a bar graph showing effect of 20-mer phosphorothioate oligonucleotides on PKC- $\eta$  expression in A549 cells.

Figure 13 is a nucleotide sequence (SEQ ID NO: 104) of a portion of the 3' untranslated region of the human PKC $\alpha$  gene beginning at the Bcl I site near the 3' end of the previously known sequence and extending in the 3' direction. Newly determined sequences begin at nucleotide 56 and are underlined (SEQ ID NO:105). Bold sequences are unique to the long mRNA transcript of PKC $\alpha$  (SEQ ID NO:106).

Figure 14 is a line graph showing a time course of PKC $\alpha$  mRNA levels in cells (shown as percent of control) after treatment with oligonucleotide 7911 (SEQ ID NO: 117). Levels of both the short and long mRNA transcripts are indicated. Levels of short mRNA transcript are represented by solid lines. Levels of long mRNA transcript are represented by dotted lines. By 12 hours after treatment with ISIS 7911 (SEQ ID NO: 117), levels of both messages were reduced by over 80%.

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**SUMMARY OF THE INVENTION**

In accordance with the present invention, oligonucleotides are provided that are specifically hybridizable with DNA or RNA deriving from the gene that encodes PKC. The oligonucleotide comprises nucleotide units sufficient in identity and number to effect such specific hybridization. This relationship is commonly denominated as "antisense". In one preferred embodiment, the oligonucleotides are specifically hybridizable with the translation initiation codon of the gene, and preferably comprise a sequence CAT. In another preferred embodiment, the oligonucleotides are specifically hybridizable with the 5'-untranslated or 3'-untranslated regions of the gene. In yet another preferred embodiment, oligonucleotides are provided that are specifically hybridizable with DNA or mRNA encoding a particular PKC isozyme or a particular set of PKC isozymes. Such oligonucleotides may be conveniently and desirably presented in a pharmaceutically acceptable carrier.

In accordance with other preferred embodiments, the oligonucleotides comprise one or more chemical modifications which convey some desired characteristic such as improved target affinity, cellular uptake or stability in the presence of cellular nucleases. Examples of modifications having such utility are 2'-O-alkyl and 2'-fluoro sugar modifications and phosphorothioate backbone modifications.

Other aspects of the invention are directed to methods for modulating the expression of PKC or of a particular PKC isozyme or set of isozymes in cells or tissues. Additional aspects of the invention are directed to methods of detection in cells or tissues of the DNA or RNA that encodes PKC and specific detection in cells or tissues of RNA or DNA that encodes particular PKC isozymes. Such methods comprise contacting cells or tissues suspected of containing said gene with oligonucleotides in accordance

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with the invention in order to interfere with the effect of or to detect said RNA or DNA.

Other aspects of the invention are directed to methods for diagnostics and therapeutics of animals

- 5 suspected of having a disease associated with PKC or one of its isozymes. Such methods comprise contacting the animal or cells or tissues or a bodily fluid from the animal with oligonucleotides in accordance with the invention in order to modulate the expression of PKC, to treat conditions  
10 associated with PKC, or to effect a diagnosis thereof.

This invention provides nucleic acid sequences that encode portions of the 3' untranslated region of human PKC $\alpha$ . Polynucleotide probes and methods of detecting PKC $\alpha$  are also provided. In some embodiments of the present  
15 invention, nucleic acid sequences specific for a particular mRNA transcript of PKC $\alpha$  are provided, as well as polynucleotide probes and methods for specific detection of this transcript.

In accordance with other embodiments of the present  
20 invention, antisense oligonucleotides are provided that are specifically hybridizable with nucleic acids encoding PKC $\alpha$ . In still other embodiments, antisense oligonucleotides are provided which are specifically hybridizable with a particular mRNA transcript of PKC $\alpha$ . Such oligonucleotides  
25 may be conveniently and desirably presented in a pharmaceutically acceptable carrier.

In accordance with still other aspects of the invention are provided methods for modulating the expression of PKC $\alpha$  or of a particular PKC $\alpha$  mRNA transcript  
30 in cells. Additional aspects of the invention are directed to methods of detection in cells of nucleic acids that encode PKC $\alpha$  and specific detection in cells of nucleic acids that encode particular PKC $\alpha$  transcripts. Such methods comprise contacting the cells with oligonucleotides in  
35 accordance with the invention in order to interfere with the effect of or to detect said nucleic acid.

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In still other embodiments of the invention are provided methods for treating animals having a disease associated with expression of PKC $\alpha$  or one of its transcripts. Such methods comprise contacting the animal with a therapeutically effective amount of oligonucleotides in accordance with the invention in order to modulate the expression of PKC $\alpha$ , to treat conditions associated with PKC $\alpha$ , or to effect a diagnosis thereof.

#### DETAILED DESCRIPTION OF THE INVENTION

Antisense oligonucleotides are now accepted as therapeutic agents having promise for the treatment of many human diseases. Oligonucleotides specifically bind (hybridize) to the complementary sequence of DNA, pre-mRNA or mature mRNA, as defined by Watson-Crick base pairing, interfering with the flow of genetic information from DNA to protein. The properties of antisense oligonucleotides which make them specific for their target sequence also make them extraordinarily versatile. Because antisense oligonucleotides are long chains of monomeric units, they may be readily synthesized for any target RNA sequence. Numerous recent studies have documented the utility of antisense oligonucleotides as biochemical tools for studying target proteins (Rothenberg et al., *J. Natl. Cancer Inst.*, 81:1539-1544 (1989); Zon, G., *Pharmaceutical Res.*, 5:539-549 (1988). Because of recent advances in oligonucleotide chemistry and synthesis of oligonucleotides which exhibit enhanced cell uptake, target binding affinity and nuclease resistance, it is now possible to consider the use of antisense oligonucleotides as a novel form of therapeutics. For example, antisense oligonucleotides targeted to c-myc have been used to completely eliminate myeloid leukemia cells from bone marrow derived from patients with acute myelogenous leukemia. Gewirtz and Calabretta, U.S. Patent 5,098,890. An antisense oligonucleotide has been shown to have clinical efficacy in

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humans for treatment of cytomegalovirus retinitis infections.

Antisense oligonucleotides offer an ideal solution to the problems encountered in prior art approaches to the treatment of conditions associated with PKC. They can be designed to selectively inhibit a given isozyme or particular set of isozymes, or to inhibit all members of a given family of isozymes.

Current agents which modulate the activity or metabolism of protein kinase C exhibit many unacceptable side effects due to their lack of specificity, or they exhibit only limited effectiveness in inhibiting the enzyme. The instant invention circumvents problems encountered by prior workers by modulating the production of the enzyme, rather than inhibiting the enzyme directly, to achieve the therapeutic effect. In the instant invention, the oligonucleotide is designed to hybridize directly to mRNA or to a gene, ultimately modulating the amount of PKC protein made from the gene. "Hybridization," in the context of this invention, means hydrogen bonding, also known as Watson-Crick base pairing, between complementary bases, usually on opposite nucleic acid strands or two regions of a nucleic acid strand, to form a double-stranded duplex. Guanine and cytosine are examples of complementary bases which are known to form three hydrogen bonds between them. Adenine and thymine are examples of complementary bases which are known to form two hydrogen bonds between them. "Specifically hybridizable" and "substantially complementary" are terms which indicate a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide (or polynucleotide probe) to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays and therapeutic treatment, or, in the case of *in vitro* assays, under conditions in which the assays are conducted. It is understood that an oligonucleotide or polynucleotide probe

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need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable.

The relationship between an oligonucleotide and its complementary (or "target") nucleic acid is commonly  
5 denoted as "antisense."

It is preferred to target specific genes for antisense attack. It has been discovered that the genes coding for PKC  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$  and  $\eta$  are particularly useful for this approach. Inhibition of PKC expression is  
10 expected to be useful for the treatment of diseases, particularly hyperproliferative and inflammatory disorders. However, "modulation" in the context of this invention means either an increase or decrease (stimulation or inhibition) of PKC expression.

15 In the context of this invention, the term "oligonucleotide" refers to a polynucleotide formed from naturally occurring nucleobases and pentofuranosyl (sugar) groups joined by native phosphodiester bonds. This term effectively refers to naturally occurring species or  
20 synthetic species formed from naturally occurring subunits or their close homologs.

The term "oligonucleotide" may also refer to moieties which function similarly to naturally occurring oligonucleotides but which have non-naturally occurring  
25 portions. Thus, oligonucleotides may have altered sugar moieties, nucleobases or inter-sugar ("backbone") linkages. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced cellular uptake, enhanced target  
30 binding affinity and increased stability in the presence of nucleases.

Specific examples of some preferred oligonucleotides envisioned for this invention are those which contain intersugar backbone linkages such as phosphotriesters,  
35 methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are those

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with  $\text{CH}_2\text{-NH-O-CH}_2$ ,  $\text{CH}_2\text{-N(CH}_3\text{)-O-CH}_2$ ,  $\text{CH}_2\text{-O-N(CH}_3\text{)-CH}_2$ ,  $\text{CH}_2\text{-N(CH}_3\text{)-N(CH}_3\text{)-CH}_2$  and  $\text{O-N(CH}_3\text{)-CH}_2\text{-CH}_2$  backbones (where phosphodiester is  $\text{O-P-O-CH}_2$ ). Phosphorothioates are also most preferred. Also preferred are oligonucleotides having

5 morpholino backbone structures. Summerton, J.E. and Weller, D.D., U.S. Patent 5,034,506. In other preferred embodiments, such as the peptide nucleic acid (PNA - referred to by some as "protein nucleic acid") backbone, the phosphodiester backbone of the oligonucleotide may be

10 replaced with a polyamide backbone wherein nucleosidic bases are bound directly or indirectly to aza nitrogen atoms or methylene groups in the polyamide backbone. *see, e.g.,* P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt, *Science* 1991, 254, 1497 and United States Patent

15 Application Serial No. 08/054,363, filed April 26, 1993 and incorporated herein by reference. In accordance with other preferred embodiments, the phosphodiester bonds are substituted with structures which are chiral and enantiomerically specific. Persons of ordinary skill in

20 the art will be able to select other linkages for use in practice of the invention.

Oligonucleotides may also include species which include at least one modified nucleobase. Thus, purines and pyrimidines other than those normally found in nature

25 may be so employed. Similarly, modifications on the pentofuranosyl portion of the nucleotide subunits may also be effected, as long as the essential tenets of this invention are adhered to. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides.

30 Some specific examples of modifications at the 2' position of sugar moieties which are useful in the present invention are OH, SH,  $\text{SCH}_3$ , F, OCN,  $\text{O(CH}_2\text{)}_n\text{NH}_2$  or  $\text{O(CH}_2\text{)}_n\text{CH}_3$  where n is from 1 to about 10;  $\text{C}_1$  to  $\text{C}_{10}$  lower alkyl, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN;  $\text{CF}_3$ ;  $\text{OCF}_3$ ; O-, S-, or

35 N-alkyl; O-, S-, or N-alkenyl;  $\text{SOCH}_3$ ;  $\text{SO}_2\text{CH}_3$ ;  $\text{ONO}_2$ ;  $\text{NO}_2$ ;  $\text{N}_3$ ;  $\text{NH}_2$ ; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group;



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a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. One or more pentofuranosyl groups may be replaced by another sugar, by a sugar mimic such as cyclobutyl or by another moiety which takes the place of the sugar.

Chimeric or "gapped" oligonucleotides are also preferred embodiments of the invention. These oligonucleotides contain two or more chemically distinct regions, each comprising at least one nucleotide. Typically, one or more region comprises modified nucleotides that confer one or more beneficial properties, for example, increased nuclease resistance, increased uptake into cells or increased binding affinity for the RNA target. One or more unmodified or differently modified regions retain the ability to direct Rnase H cleavage. Chimeric oligonucleotides are disclosed in PCT application US92/11339 which is assigned to the assignee of the instant application and which is incorporated by reference herein in its entirety. Examples of chimeric oligonucleotides which are presently preferred are 2'-O-methyl or 2'-O-propyl oligonucleotides having a "deoxy gap" region of 2'-deoxynucleotides. Usually this deoxy gap region is located between the two 2'-alkyl regions. In these preferred embodiments, the internucleotide (backbone) linkages may be uniformly phosphorothioate or some combination of phosphorothioate and phosphodiester linkages.

All such oligonucleotides are best described as being functionally interchangeable with natural oligonucleotides (or synthesized oligonucleotides along natural lines), but having one or more differences from natural structure. All such oligonucleotides are comprehended by this invention so long as they function effectively to hybridize with the PKC RNA.

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The oligonucleotides in accordance with this invention preferably comprise from about 5 to about 50 nucleotide units. It is more preferred that such oligonucleotides comprise from about 8 to 30 nucleotide units, and still more preferred to have from about 12 to 25 nucleotide units. As will be appreciated, a nucleotide unit is a base-sugar combination (or a combination of analogous structures) suitably bound to an adjacent nucleotide unit through phosphodiester or other bonds forming a backbone structure.

The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotides such as phosphorothioates or alkylated derivatives. Other modified and substituted oligomers can be similarly synthesized.

In accordance with this invention, persons of ordinary skill in the art will understand that messenger RNA includes not only the coding region, which contains information to encode a protein using the three letter genetic code, but also associated ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region, the 5' cap region and intron/exon junction ribonucleotides. Thus, oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the coding ribonucleotides. In preferred embodiments, the oligonucleotide is specifically hybridizable with a transcription initiation site, a translation initiation site, a 5' cap region, an intron/exon junction, coding

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sequences or sequences in the 5'- or 3'-untranslated region.

The oligonucleotides of this invention are designed to be hybridizable with the PKC gene or with  
5 messenger RNA derived from the PKC gene. Such hybridization, when accomplished, interferes with the normal roles of the messenger RNA to cause a modulation of its function in the cell. The functions of messenger RNA to be interfered with may include all vital functions such  
10 as translocation of the RNA to the site for protein translation, actual translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and possibly even independent catalytic activity which may be engaged in by the RNA. The overall effect of such  
15 interference with the RNA function is to modulate expression of the PKC gene.

The oligonucleotides of this invention can be used in diagnostics, therapeutics, prophylaxis, and as research reagents and kits. Since the oligonucleotides of this  
20 invention hybridize to the PKC gene and its mRNA, sandwich and other assays can easily be constructed to exploit this fact. Furthermore, since the oligonucleotides of this invention hybridize specifically to particular isozymes of the PKC mRNA, such assays can be devised for screening of  
25 cells and tissues for particular PKC isozymes. Such assays can be utilized for diagnosis of diseases associated with various PKC forms. Provision of means for detecting hybridization of oligonucleotide with the PKC gene can routinely be accomplished. Such provision may include  
30 enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of PKC may also be prepared.

For therapeutic or prophylactic treatment, oligonucleotides are administered in accordance with this  
35 invention. Oligonucleotides may be formulated in a pharmaceutical composition, which may include carriers, thickeners, diluents, buffers, preservatives, surface

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- active agents and the like in addition to the oligonucleotide. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like in addition to oligonucleotides.

- The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be done topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip or subcutaneous, intraperitoneal or intramuscular injection.

- Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms may also be useful.

- Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

- Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

- Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

- The present invention also provides a nucleic acid molecule having a sequence which encodes the 3'-untranslated region of human PKC $\alpha$  is provided (Figure 13).

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This sequence was determined from cDNA clones prepared from human A549 cells, beginning with a clone overlapping the 3'-most end of the previously published PKC $\alpha$  sequence [Finkenzeller et al., *Nucl. Acids Res.* 18:2183 (1990); Genbank accession number X52479] and extending in the 3' direction. A polyadenylation site which was reached after 1080 nucleotides (nucleotide 1136 in Figure 13); has been identified as the 3' end of the short (4 kb) mRNA transcript of PKC $\alpha$ . An additional 676 nucleotides of sequence in the 3' direction were determined, which sequence is unique to the long (8kb) mRNA transcript of PKC $\alpha$ . The nucleic acid molecule of the present invention may preferably be comprised of deoxyribonucleic acids and may be double-stranded in some aspects of the present invention. Also in accordance with the present invention, said nucleic acid molecules are isolated. "Isolated" as the term is used herein, is meant to refer to molecules which have been purified or synthesized so as to be substantially homogenous. The term does not exclude the possibility that certain impurities may be present in the composition, but is, instead, meant to refer to the absence of non-relevant nucleic acid sequences.

In accordance with the present invention polynucleotide probes specifically hybridizable to a portion of the 3' untranslated region of the human PKC $\alpha$  gene are provided. Polynucleotide probes specifically hybridizable to a portion of the long mRNA transcript of PKC $\alpha$  are also provided. Such probes may be used for diagnostic or research purposes to detect or quantitate the expression of PKC $\alpha$ . Probes may be used to specifically detect or quantitate the long transcript of PKC $\alpha$ . Said polynucleotide probes may range in length from about 5 to about 50 nucleotide units. In more preferred embodiments of the present invention the probes may be from about 8 to about 30 nucleotide units in length. Ideally, said probes range in length from about 12 to about 25 nucleotide units. It is recognized that since polynucleotide probes of the

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present invention ideally do not exceed 50 nucleotides in length, said probes may specifically hybridize to only a portion of the targeted sequence. The portion of the PKC $\alpha$  sequence to be targeted can be identified by one skilled in the art. Most suitably, a target sequence is chosen which is unique, thereby decreasing background noise attributable to hybridization by the probe other than to the target. By way of example, one skilled in the art would be unlikely to select a repeating sequence of adenine nucleotide units as this is a common sequence occurring in many genes. The practitioner might choose to perform a search and comparison of sequences found in a sequence depository such as Genbank in order to identify and design a useful probe. Such methods are conventionally used to identify unique sequences. These unique sequences, when used as probes, need not necessarily be crucial to the regulation of the expression of PKC $\alpha$ .

The following examples illustrate the present invention and are not intended to limit the same.

## 20 EXAMPLES

### Example 1 Oligonucleotide synthesis:

Unmodified DNA oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.  $\beta$ -cyanoethyl-diisopropyl-phosphoramidites were purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step was increased to 68 seconds and was followed by the capping step.

2'-O-methyl phosphorothioate oligonucleotides were synthesized according to the procedures set forth above substituting 2'-O-methyl  $\beta$ -cyanoethyl-diisopropyl phosphoramidites (Chemgenes, Needham, MA) for standard

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phosphoramidites and increasing the wait cycle after the pulse delivery of tetrazole and base to 360 seconds. Similarly, 2'-O-propyl phosphorothioate oligonucleotides may be prepared by slight modifications of this procedure.

- 5 After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides were purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Analytical gel  
10 electrophoresis was accomplished in 20% acrylamide, 8 M urea, 45 mM Tris-borate buffer, Ph 7.0.

- The oligonucleotides tested are presented in Table 1. Sequence data are from the cDNA sequence published by Finkenzeller et al., *Nucl. Acids Res.* 18:2183 (1990);  
15 Genbank accession number X52479. The sequence numbers given under the oligonucleotides are relative to the first residue to be sequenced on the cDNA, which is 28 residues upstream of the ATG start codon.

Table 1

OLIGONUCLEOTIDES TARGETED TO HUMAN PKC- $\alpha$			
SEQ ID	Sequence	Target	ISIS #
20 1	CCC CAA CCA CCT CTT GCT CC 19 1	5' Untranslated	3520
25 2	GTT CTC GCT GGT GAG TTT CA 2063 2044	3' Untranslated	3521
3	AAA ACG TCA GCC ATG GTC CC 41 22	Translation init. codon	3522
4	GGA TTC ACT TCC ACT GCG GG 2109 2090	3' Untranslated	3526
30 5	GAG ACC CTG AAC AGT TGA TC 2211 2192	3' Untranslated	3527
6	CCC GGG AAA ACG TCA GCC AT 47 28	Translation init codon	3674
35 7	CTG CCT CAG CGC CCC TTT GC 110 91	Internal (C1) domain	3682

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	8	AGT CGG TGC AGT GGC TGG AG	Internal	3686
		193 174	(C1) domain	
	9	GCA GAG GCT GGG GAC ATT GA	Internal	3687
		480 461	(C1) domain	
5	10	GGG CTG GGG AGG TGT TTG TT	3'	3695
		2080 2061	Untranslated	
	11	CAC TGC GGG GAG GGC TGG GG	3'	3875
		2098 2079	Untranslated	
10	12	AGC CGT GGC CTT AAA ATT TT	3'	3878
		2137 2118	Untranslated	
	13	ATT TTC AGG CCT CCA TAT GG	3'	3879
		2168 2149	Untranslated	
	14	AAG AGA GAG ACC CTG AAC AG	3'	3884
		2217 2198	Untranslated	
15	15	GAT AAT GTT CTT GGT TGT AA	3'	3885
		2235 2216	Untranslated	
	16	ATG GGG TGC ACA AAC TGG GG	Internal	3886
		2027 2008	(C3) domain	
20	17	GTC AGC CAT GGT CCC CCC CC	Translation	3890
		36 17	init. codon	
	18	CGC CGT GGA GTC GTT GCC CG	Internal	3891
		63 44	(V1) domain	
	19	TCA AAT GGA GGC TGC CCG GC	Internal	3892
		1643 1624	(C3) domain	
25	20	TGG AAT CAG ACA CAA GCC GT	3'	3947
		2151 2132	Untranslated	

**Example 2 Cell culture and treatment with phorbol esters and oligonucleotides targeted to PKC- $\alpha$ :**

PKC protein half-lives have been reported to vary from 6.7 hours to over 24 hours [Young et al., *Biochem. J.* 244:775-779 (1987); Ballester et al., *J. Biol. Chem.* 260:15194-15199 (1985)]. These long half-lives make inhibiting steady-state levels of PKC- $\alpha$  an unwieldy approach when screening antisense oligonucleotides, due to the long incubation times which would be required. We have therefore made use of the ability of phorbol esters to reversibly lower intracellular levels of PKC. Treatment of



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cells with phorbol esters causes an initial activation of kinase activity, followed by a down-regulation of PKC. For PKC- $\alpha$  this down-regulation has been shown to be a direct consequence of an increased rate of proteolysis of the kinase with no apparent change in synthetic rate.

- We determined that in human lung carcinoma (A549) cells, treatment with the phorbol ester 12,13-dibutyrate (PDBu), using a modification of the method of Krug et al., [Krug et al., *J. Biol. Chem.* 262:11852-11856 (1987)] lowered cellular levels of PKC- $\alpha$ , without affecting PKC- $\alpha$  mRNA levels, and that this effect was reversible. The basis of the assay to screen for potency of oligonucleotides targeting PKC- $\alpha$  is to initially lower PKC- $\alpha$  protein levels by chronic treatment with PDBu, remove PDBu by extensively washing the cells (hence allowing the cells to synthesize fresh PKC- $\alpha$  protein), and incubate the cells with oligonucleotides intended to inhibit the resynthesis of new PKC- $\alpha$  protein.

- Procedure: A549 cells (obtained from the American Type Culture Collection, Bethesda MD) were grown to confluence in 6-well plates (Falcon Labware, Lincoln Park, NJ) in Dulbecco's modified Eagle's medium (DME) containing 1 g glucose/liter and 10% fetal calf serum (FCS, Irvine Scientific, Santa Ana, CA).

- Cells were treated with 500 nM PDBu (Sigma Chem. Co., St. Louis, MO) for 12-16 hours (overnight). Cells were then washed three times in DME at 37°C, and 1 ml DMA containing 20  $\mu$ l DOTMA (Lipofectin reagent, BRL, Bethesda, MD) was added. Oligonucleotides were added to a concentration of 1  $\mu$ M and the cells were incubated for a further 4 hours at 37°C.

- Cells were washed once in 3 ml DME containing 0.1 mg/ml BSA and a further 2 ml DME containing 0.1 mg/ml BSA was added. Oligonucleotides (1  $\mu$ M) were added and the cells were incubated at 37°C for 24 hours.

Cells were washed three times in phosphate-buffered saline (PBS) and cellular proteins were extracted in 120  $\mu$ l

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sample buffer (60 mM Tris pH 6.8, 2% SDS, 10% glycerol, 10 mM dithiothreitol) and boiled for 5 minutes. Intracellular levels of PKC- $\alpha$  protein were determined by immunoblotting.

**Example 3      Immunoblot assay for PKC expression:**

5      Cell extracts were electrophoresed on 10% SDS-PAGE mini-gels. The resolved proteins were transferred to Immobilon-P membrane (Millipore, Bedford MA) by electrophoretic transfer and the membrane was blocked for 60 minutes in TBS (Tris-HCl pH 7.4, 150 mM NaCl) containing 5% nonfat milk. The membrane was then incubated for 16  
10      hours at 4°C with monoclonal antibodies raised against PKC- $\alpha$  (UBI, Lake Placid NY) diluted to 0.2  $\mu$ g/ml in TBS containing 0.2% nonfat milk. This was followed by three washes in TBS plus 0.2% nonfat milk. The membrane was then  
15      incubated for one hour with <sup>125</sup>I-labelled goat anti-mouse secondary antibody (ICN Radiochemicals, Irvine CA). Membranes were then washed extensively in TBS plus 0.2% nonfat milk. Bands were visualized and quantitated using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). PKC- $\alpha$   
20      appears as a single band with a molecular weight of 80 kD.

Each oligonucleotide was tested three times, in triplicate, and the results of the experiments were normalized against percentage of protein present as compared to cells which were not treated with  
25      oligonucleotide (Figures 1a and 1b). The five most effective oligonucleotides target the AUG start codon and regions slightly upstream and downstream from it (Sequence Nos. 1, 3, 17, 7, 6). The next most effective oligonucleotides are targeted toward the 3' untranslated region  
30      of the RNA (oligos 2, 5, 14).

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**Example 4 Other isozymes of PKC:**

Results with oligonucleotides targeting human PKC- $\alpha$  demonstrated that the most effective target sequences were those surrounding the translation initiation codon and the 3' untranslated region. It is believed that these sequences will also be effective targets for oligonucleotides directed against other isozymes of PKC. Antisense oligonucleotides which are likely to be effective inhibitors of PKC are identified below. These oligonucleotides are synthesized as in Example 1, and can be screened as in Examples 2 and 3, using appropriate antibodies where available. Alternatively, a reporter gene assay system can be established, transiently co-expressing the desired isozyme of PKC with luciferase under the influence of the TPA-responsive enhancer or other suitable promoter. PKC expression is then assayed by measuring luciferase activity using standard procedures. Luciferase is extracted from cells by lysis with the detergent Triton X-100, as described by Greenberg, M.E., in *Current Protocols in Molecular Biology*, (F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.A. Smith, J.G. Seidman and K. Strahl, eds.), John Wiley and Sons, NY (1987). A Dynatech ML1000 luminometer is used to measure peak luminescence upon addition of luciferin (Sigma) to 625  $\mu$ M.

**25 PKC- $\beta$ , types I and II**

Sequence data are from Kubo et al., *FEBS Lett.* 223: 138-142 (1987); Genbank accession numbers X06318, M27545, X07109. Sequences are numbered from the first 5' base sequenced on the cDNA. PKC- $\beta$  types I and II are the result of alternative mRNA splicing of a single gene product. This results in proteins with identical amino termini (5' end of the mRNA); however, there is sequence divergence in the carboxy termini (3' end of the mRNA). The following oligonucleotides, targeted to the translation initiation codon, are expected to modulate expression of both PKC- $\beta$  types I and II:

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TABLE 2

OLIGONUCLEOTIDES TARGETED TO PKC- $\beta$  TYPES I AND II

SEQ ID	Sequence	Target
5 21	CAT CTT GCG CGC GGG GAG CC 139 120	Translation init.
22	TGC GCG CGG GGA GCC GGA GC 134 115	" "
23	CGA GAG GTG CCG GCC CCG GG 113 94	" "
10 24	CTC TCC TCG CCC TCG CTC GG 183 164	" "

The following antisense oligonucleotides are targeted to the 3'-untranslated region of PKC- $\beta$  type I:

TABLE 3

15 OLIGONUCLEOTIDES TARGETED TO PKC- $\beta$  TYPE I

SEQ.ID	Sequence	Target
25	TGG AGT TTG CAT TCA CCT AC 2168 2149	3' Untranslated
20 26	AAA GGC CTC TAA GAC AAG CT 2285 2266	" "
27	GCC AGC ATG TGC ACC GTG AA 2250 2231	" "
28	ACA CCC CAG GCT CAA CGA TG 2186 2167	" "
25 29	CCG AAG CTT ACT CAC AAT TT 2569 2550	" "

The following antisense oligonucleotides are targeted to the 3'-untranslated region of PKC- $\beta$  Type II:

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TABLE 4

OLIGONUCLEOTIDES TARGETED TO PKC- $\beta$  TYPE II

	SEQ. ID	Sequence	Target
5	30	ACT TAG CTC TTG ACT TCG GG 2160 2141	3' Untranslated
	31	ATG CTG CGG AAA ATA AAT TG 2420 2401	" "
	32	ATT TTA TTT TGA GCA TGT TC 2663 2644	" "
10	33	TTT GGG GAT GAG GGT GAG CA 2843 2824	" "
	34	CCC ATT CCC ACA GGC CTG AG 3137 3118	" "

PKC- $\gamma$ :

- 15 Sequence data are from Coussens et al., *Science* 233:859-866 (1986); Genbank accession number M13977. Sequences are numbered from the first 5' base sequenced in the cDNA. The full sequence is not available: the extreme 3' end of the open reading frame and the 3' untranslated
- 20 region are missing. Consequently these regions are not presently available as antisense targets.

TABLE 5

OLIGONUCLEOTIDES TARGETED TO PKC- $\gamma$ 

	SEQ.ID	Sequence	Target
25	35	CGG AGC GCG CCA GGC AGG GA 51 32	5' Untranslated
	36	CCT TTT CCC AGA CCA GCC AT 215 196	Translation init.
	37	GGC CCC AGA AAC GTA GCA GG 195 176	5' of start codon
30	38	GGA TCC TGC CTT TCT TGG GG 170 151	5' Untranslated
	39	CAG CCA TGG CCC CAG AAA CG 202 183	Translation init.

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**PKC- $\eta$ :**

Sequence data for PKC- $\eta$  are from Bacher and colleagues [Bacher et al., *Mol. Cell. Biol.* 11:126-133 (1991)]; Genbank accession number M55284. They assign their 5 isozyme the name PKC-L; however the sequence is almost identical to that of mouse PKC- $\eta$ , so the latter nomenclature is used here for consistency. Sequences are numbered from the first 5' base sequenced in the cDNA.

**TABLE 6**

10		OLIGONUCLEOTIDES TARGETED TO PKC- $\eta$	
	SEQ.ID	Sequence	Target
	40	CGA CAT GCC GGC GCC GCT GC 172 153	Translation init.
	41	CAG ACG ACA TGC CGG CGC CG 176 157	" "
15	42	GCC TGC TTC GCA GCG GGA GA 138 119	" "
	43	ACA GGT GCA GGA GTC GAG GC 86 67	" "
20	44	GTC CCG TCT CAG GCC AGC CC 111 92	" "
	45	CCT CAC CGA TGC GGA CCC TC 221 202	" "
	46	ATT GAA CTT CAT GGT GCC AG 193 174	" "
25	47	TCT CAC TCC CCA TAA GGC TA 2046 2027	3' Untranslated
	48	TTC CTT TGG GTT CTC GTG CC 2067 2048	" "
30	49	TTC CAT CCT TCG ACA GAG TT 2353 2336	" "
	50	AGG CTG ATG CTG GGA AGG TC 2300 2281	" "
35	51	GTT CTA AGG CTG ATG CTG GG 2306 2287	" "

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**Example 5      Dose response of phosphorothioate/2'-O-methyl oligonucleotide effects on PKC- $\alpha$  protein synthesis:**

- A series of phosphorothioate, fully 2'-O-methyl oligonucleotides having SEQ ID NO: 1, 2, 3 and 5 were synthesized. A549 cells were treated with 500 nM PDBu for 18 hours to downregulate PKC- $\alpha$  synthesis, washed to remove PDBu and then treated with oligonucleotide and DOTMA/DOPE cationic liposomes. Medium was replaced after four hours and the cells were allowed to recover for another 20 hours.
- 10 Proteins were extracted and PKC- $\alpha$  protein levels were determined by immunoblotting as described in Example 3. Results were quantified with a phosphorimager (Molecular Dynamics, Sunnyvale CA) and are shown in Figure 2 expressed as percent of control (saline treatment). ISIS 4649 (SEQ ID
- 15 NO: 3; squares) reduced PKC- $\alpha$  protein levels by 85-90% at 500 nM and had an IC50 of approximately 260 nM.

**Example 6      Effect of antisense oligonucleotides on PKC- $\alpha$  mRNA levels:**

- A549 cells were treated with phosphorothioate oligonucleotides at 500 nM for four hours in the presence of the cationic lipids DOTMA/DOPE, washed and allowed to recover for an additional 20 hours. Total RNA was extracted and 20 $\mu$ g of each was resolved on 1.2% gels and transferred to nylon membranes. These blots were probed with a <sup>32</sup>P
- 20 radiolabeled PKC- $\alpha$  cDNA probe and then stripped and reprobed with a radiolabeled G3PDH probe to confirm equal RNA loading. Each oligonucleotide (3520, 3521, 3522 and 3527) was used in duplicate. The two major PKC- $\alpha$  transcripts (8.5 kb and 4.0 kb) were examined and
- 25 quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale CA). Results are shown in Figure 3. Oligonucleotides 3521 (SEQ ID NO: 2), 3522 (SEQ ID NO: 3) and 3527 (SEQ ID NO: 5) gave better than 50% reduction of PKC- $\alpha$  mRNA levels. Oligonucleotides 3521 and 3527 gave
- 30 approximately 80% reduction of the smaller transcript and over 90% reduction of the larger transcript.

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**Example 7      Chimeric (deoxy gapped) 2'-O-methyl  
oligonucleotides:**

Oligonucleotides 3521 (SEQ ID NO: 2), 3522 (SEQ ID NO: 3) and 3527 (SEQ ID NO: 5) were chosen for further study and modification. Oligonucleotides having these sequences were synthesized as uniformly phosphorothioate chimeric oligonucleotides having a centered deoxy gap of various lengths flanked by 2'-O-methylated regions. These oligonucleotides (500 nM concentration) were tested for effects on PKC- $\alpha$  mRNA levels by Northern blot analysis. Results are shown in Figure 4. Deoxy gaps of eight nucleotides or more gave maximal reduction of PKC- $\alpha$  mRNA levels (both transcripts) in all cases. The oligonucleotide having SEQ ID NO: 3 reduced PKC- $\alpha$  mRNA by approximately 83% with a deoxy gap length of four nucleotides, and gave nearly complete reduction of PKC- $\alpha$  mRNA with a deoxy gap length of six or more.

Dose-response curves for these oligonucleotides are shown in Figure 5. The 2'-O-methyl chimeric oligonucleotides with four- or six-nucleotide deoxy gaps have an IC<sub>50</sub> for PKC- $\alpha$  mRNA reduction (concentration of oligonucleotide needed to give a 50% reduction in PKC- $\alpha$  mRNA levels) of 200-250 nM, as did the full-deoxy oligonucleotide (all are phosphorothioates throughout). The 2'-O-methyl chimeric oligonucleotide with an 8-nucleotide deoxy gap had an IC<sub>50</sub> of approximately 85 nM.

Several variations of this chimeric oligonucleotide (SEQ. ID NO: 3) were compared for ability to lower PKC- $\alpha$  mRNA levels. These oligonucleotides are shown in Table 7.



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Table 7

Chimeric 2'-O-methyl/deoxy P=S oligonucleotides

bold= 2'-O-methyl; s= P=S linkage, o= P=O linkage

OLIGO #	SEQUENCE	SEQ ID NO:
5 3522	<b>AsAsAsAsCsGsTsCsAsGsCsCsAsTsGsGsTsCsCsC</b>	3
5352	<b>AsAsAsAsCsGsTsCsAsGsCsCsAsTsGsGsTsCsCsC</b>	3
6996	<b>AoAoAoAoCoGsTsCsAsGsCsCsAsTsGoGoToCoCoC</b>	3
7008	<b>AsAoAoAoCoGsTsCsAsGsCsCsAsTsGoGoToCoCoC</b>	3
7024	<b>AsAoAoAoCoGsToCsAoGsCoCsAsTsGoGoToCoCoC</b>	3

- 10 Effects of these oligonucleotides on PKC- $\alpha$  mRNA levels is shown in Figure 6. Oligonucleotides 7008, 3522 and 5352 show reduction of PKC- $\alpha$  mRNA, with 5352 being most active.

A series of 2'-O-propyl chimeric

oligonucleotides was synthesized having SEQ ID NO: 3. These

- 15 oligonucleotides are shown in Table 8.

Table 8

Chimeric 2'-O-propyl/deoxy P=S oligonucleotides

bold= 2'-O-propyl; s= P=S linkage, o= P=O linkage

OLIGO #	SEQUENCE	SEQ ID NO:
20 7199	<b>AsAsAsAsCsGsTsCsAsGsCsCsAsTsGsGsTsCsCsC</b>	3
7273	<b>AoAoAoAoCoGsTsCsAsGsCsCsAsTsGoGoToCoCoC</b>	3
7294	<b>AsAoAoAoCoGsTsCsAsGsCsCsAsTsGoGoToCoCoC</b>	3
7295	<b>AsAoAoAoCoGsToCsAoGsCoCsAsTsGoGoToCoCoC</b>	3

These 2'-O-propyl chimeric oligonucleotides were compared

- 25 to the 2'-O-methyl chimeric oligonucleotides. Oligonucleotides 7273 and 7294 were more active than their 2'-O-methyl counterparts at lowering PKC- $\alpha$  mRNA levels. This is shown in Figures 7 and 8.

**Example 8 Additional oligonucleotides which decrease**

- 30 **PKC- $\alpha$  mRNA:**

Additional phosphorothioate oligonucleotides targeted to the human PKC- $\alpha$  3' untranslated region were

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designed and synthesized. These sequences are shown in Table 9.

Table 9

		Chimeric 2'-O-propyl/deoxy P=S oligonucleotides targeted to PKC- $\alpha$ 3'-UTR						
		bold= 2'-O-propyl; s= P=S linkage, o= P=O linkage						
		OLIGO #	SEQUENCE					SEQ ID NO:
5		6632	TsTsCs	TsCsGs	CsTsGs	GsTsGs	AsGsTs	TsTsC 52
		6653	<b>TsTsCs</b>	TsCsGs	CsTsGs	GsTsGs	AsGsTs	<b>TsTsC</b> 52
10		6665	<b>ToToCo</b>	TsCsGs	CsTsGs	GsTsGs	AsGsTo	<b>ToToC</b> 52
		7082	TsCsTs	CsGsCs	TsGsGs	TsGsAs	GsTsTs	TsC 53
		7083	<b>TsCsTs</b>	CsGsCs	TsGsGs	TsGsAs	<b>GsTsTs</b>	<b>TsC</b> 53
		7084	<b>ToCoTo</b>	CsGsCs	TsGsGs	TsGsAs	GsToTo	<b>ToC</b> 53

As shown in Figure 9, oligonucleotides 6632, 6653, 7082 and 15 7083 are most active in reducing PKC- $\alpha$  mRNA levels.

#### Example 9 Culture of human A549 lung tumor cells:

The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (Bethesda MD). Cells were grown in Dulbecco's Modified Eagle's Medium (Irvine Scientific, Irvine CA) containing 1 gm glucose/liter and 10% fetal calf serum (Irvine Scientific). Cells were trypsinized and washed and resuspended in the same medium for introduction into mice.

#### Example 10 Effect of ISIS 3521 on the growth of human A549 tumor cells in nude mice:

200  $\mu$ l of A549 cells ( $5 \times 10^6$  cells) were implanted subcutaneously in the inner thigh of nude mice. ISIS 3521, a phosphorothioate oligonucleotide with Sequence ID NO 2 was administered twice weekly for four weeks, beginning one week following tumor cell inoculation. Oligonucleotides were formulated with cationic lipids (DMRIE/DOPE) and given subcutaneously in the vicinity of the tumor. Oligonucleotide dosage was 5 mg/kg with 60 mg/kg cationic lipid. Tumor size was recorded weekly.

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As shown in Figure 10, tumor growth was almost completely inhibited in two of the three mice, and reduced compared to control in the third mouse. This inhibition of tumor growth by ISIS 3521 is statistically significant. The control oligonucleotide (ISIS 1082) is a 21-mer phosphorothioate oligonucleotide without significant sequence homology to the PKC mRNA target.

Administration of oligonucleotides to mice whose tumors had already reached detectable size had no discernable effect on subsequent tumor growth.

**Example 11      Effect of antisense oligonucleotides on growth of human MDA-MB231 tumors in nude mice:**

MDA-MB231 human breast carcinoma cells were obtained from the American Type Culture Collection (Bethesda, MD). Serially transplanted MDA-MB231 tumors were established subcutaneously in nude mice. Beginning two weeks later, oligonucleotides 3521 and 3527, a phosphorothioate oligonucleotide having Sequence ID NO. 5, in saline, were administered intravenously daily for 14 days at dosages of 60 mg/kg and 6 mg/kg. Control oligonucleotide ISIS 1082 was also administered at these doses, and a saline control was also given. Tumor growth rates were monitored for the two-week period of oligonucleotide administration. As shown in Figure 11, both PKC- $\alpha$  oligonucleotides (3521 and 3527) significantly inhibit tumor growth at dosages of 60 mg/kg and 6 mg/kg. The control oligonucleotide (ISIS 1082) also showed some reduction in tumor growth, but this effect was less than with antisense oligonucleotides even at high doses, and considerably less at the lower dose. A lower-dose study was conducted using the same oligonucleotides at 6 mg/kg and 0.6 mg/kg. At 0.6 mg/kg ISIS 3521 significantly reduced tumor growth. At this concentration, ISIS 3527 also reduced tumor growth, but this result was not statistically significant.

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Example 12      Effect of oligonucleotides on the growth of murine Lewis lung carcinoma in mice:

Serially transplanted murine Lewis lung carcinomas were established in mice. Oligonucleotides 3521 and 3527 were administered intravenously every day for 14 days at doses of 6 mg/kg and 0.6 mg/kg. Tumor growth rates were monitored for the two-week period of oligonucleotide administration. As expected, these oligonucleotides, which are targeted to human PKC sequences, had insignificant effects on the mouse-derived tumors.

Example 13      Effects of antisense oligonucleotide ISIS 4189 on endogenous PKC- $\alpha$  expression in hairless mice:

In order to study oligonucleotide effects on endogenous PKC mRNA levels in normal animals, it was necessary to employ an oligonucleotide complementary to the murine PKC- $\alpha$ . ISIS 4189 is a 20-mer phosphorothioate oligonucleotide targeted to the AUG codon of mouse PKC- $\alpha$ . This region is without homology to the human PKC sequence and the oligonucleotide has no effect on expression of PKC- $\alpha$  in human cells. ISIS 4189 has an IC<sub>50</sub> of 200 nM for mRNA reduction in C127 mouse breast epithelial cells. ISIS 4189 in saline was administered intraperitoneally to hairless mice at concentrations of 1, 10 or 100 mg/kg body weight. Injections were given daily for seven days. Tissues from liver, kidney, spleen, lung and skin were removed and PKC- $\alpha$  mRNA and protein levels were determined. Histopathological examination was also performed on liver, kidney and lung samples. ISIS 4189 at 100 mg/kg inhibited endogenous PKC- $\alpha$  mRNA levels in the mouse liver to 10-15% of control (saline) levels.

Example 14      Screening of antisense oligonucleotides complementary to human PKC- $\eta$ :

A series of 20-mer phosphorothioate oligonucleotides complementary to human PKC- $\eta$  were synthesized. These oligonucleotides were screened at a



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**Example 15 Screening of antisense oligonucleotides complementary to human PKC- $\zeta$ :**

A series of 20-mer phosphorothioate oligonucleotides complementary to human PKC- $\zeta$  were synthesized as described in Example 1. The source of the target sequence was Genbank locus HSPKCZ, accession number Z15108 (Hug, H.). These oligonucleotides were screened at a concentration of 500 nM for ability to decrease PKC- $\zeta$  mRNA levels in human A549 cells, substantially as described in Example 6 using a Northern blot assay. The oligonucleotide sequences and results of the screen are shown in Table 11.

Table 11

**INHIBITION OF mRNA EXPRESSION IN HUMAN A549 CELLS USING ANTISENSE OLIGONUCLEOTIDES COMPLEMENTARY TO PKC-Z**

15	Oligo #	Sequence	Target region	%Inhib.	Seq.ID
	9007	CGCCGCTCCCTTCCATCTTG	AUG	70	63
	9008	CCCCGTAATGCGCCTTGAGG	Coding	68	64
	9009	CTGTCCACCCACTTGAGGGT	Coding	19	65
	9010	GCTTCCTCCATCTTCTGGCT	Coding	35	66
20	9011	CGGTACAGCTTCCTCCATCT	Coding	58	67
	9012	TTGGAAGAGGTGGCCGTTGG	Coding	80	68
	9013	CCTGTTAAAGCGCTTGGCTT	Coding	71	69
	9014	TGCAGGTCAGCGGGACGAGG	Coding	41	70
	9015	GCTCTTGGGAAGGCATGACA	Coding	59	71
25	9016	TTCTTCAACCCGACCAGGAG	Coding	0	72
	9017	TTCTTCAACCCGACCAGGAG	Coding	73	73
	9018	CTCTGCCTCTGCATGTGGAA	Coding	63	74
	9019	TCCTTGCACATGCCGTAGTC	Coding	31	75
	9020	TCCACGCTGAACCGTACTC	Coding	80	76
30	9021	GGAGCGCCCGCCATCATCT	Coding	81	77
	9022	GGGCTCGCTGGTGAAGTGTG	Coding	83	78
	9023	GACGCACGCGGCCTCACACC	Stop	82	79
	9024	GGGTCAATCACGCGTGTCCA	3' UTR	70	80
	9025	TCGGAGCCGTGCCGAGCCTG	3' UTR	82	81
35	9026	CGGGCCAGGTGTGAGGGACT	3' UTR	40	82
	9027	CCGCGACGCAGGCACAGCAG	3' UTR	38	83
	9028	TGGAAACCGCATGACAGCCC	3' UTR	54	84
	9029	GGTCAGTGCATCGAGTTCTG	3' UTR	79	85

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In this experiment, oligonucleotides 9007, 9008, 9011, 9012, 9013, 9015, 9017, 9018, 9020, 9021, 9022, 9023, 9024, 9025, 9028 and 9029 showed at least 50% inhibition of mRNA levels and are presently preferred.

**5 Example 16 Screening of antisense oligonucleotides complementary to human PKC- $\epsilon$ :**

A series of 20-mer phosphorothioate oligonucleotides complementary to human PKC- $\epsilon$  were synthesized as described in Example 1. The source of the target sequence was Genbank  
 10 locus HSPKCE, accession number X65293 (Burns et al.). These oligonucleotides were screened at a concentration of 500 nM for ability to decrease PKC- $\epsilon$  mRNA levels in human A549 cells, substantially as described in Example 6 using a Northern blot assay. The oligonucleotide sequences and  
 15 results of the screen are shown in Table 12.

**Table 12**

INHIBITION OF mRNA EXPRESSION IN HUMAN A549 CELLS USING ANTISENSE OLIGONUCLEOTIDES COMPLEMENTARY TO PKC-E MRNA				
Oligo #	Sequence	Target region	%Inhib	Seq.ID
20	7933 ACTACCATGGTCGGGGCGGG	AUG	0	86
	7934 GTCCACCCGATGGCGCAGC	Coding	0	87
	7935 GTTGGCCGATGCGGAGTC	Coding	0	88
	7936 TGCAGTTGGCCACGAAGTCG	Coding	0	89
	8032 GTGGGGCATGTTGACGCTGA	Coding	0	90
25	8031 CCAGAGCAGGACCCACAGT	Coding	0	91
	7939 TCTCTCGGTTGTCAAATGA	Coding	0	92
	7940 CGGTGCTCCTCTCCTCGGTT	Coding	0	93
	7941 AGCCAAAATCCTCTTCTCTG	Coding	0	94
	7942 CATGAGGGCCGATGTGACCT	Coding	62	95
30	7943 ATCCCTTCCTTGACATCCC	Coding	4	96
	7944 CCCCAGGGCCCAACAGTCCA	Coding	42	97
	7945 AGCACCCCGAGGGCCCA	Coding	56	98
	7946 CGTACATCAGCACCCCGAGG	Coding	55	99
	7947 CCAGCCATCATCTCGTACAT	Coding	15	100
35	7948 TGCCACACAGCCAGGCGCA	Coding	55	101
	7949 TCAGGGCATCAGGTCTTCAC	Stop	0	102
	7950 CTCTCAGGGCATCAGGTCTT	Stop	0	103

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In this experiment, oligonucleotides 7942, 7944, 7945, 7946 and 7948 showed at least 40% inhibition of mRNA levels and are presently preferred.

**Example 17            DNA sequencing of the 3' untranslated  
5 region of human PKC $\alpha$**

A549 cells (obtained from the American Type Culture Collection, Bethesda MD) were grown to confluence in 6-well plates (Falcon Labware, Lincoln Park, NJ) in Dulbecco's modified Eagle's medium (DME) containing 1 g glucose/liter and 10% fetal calf serum (FCS, Irvine Scientific, Santa Ana, CA). Cells were harvested and total RNA was isolated using standard methods. Sambrook, J., Fritsch, E., and T. Maniatis (1989). Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.,  
15 Ch. 7).

cDNA was made from the RNA using the 3' RACE technique of Frohman et al. [Frohman, M.A., Dush, M.K. and G.R. Martin (1988) Proc. Natl. Acad. Sci. U.S.A. 85:8998-9002] and the 3' RACE kit from Gibco/BRL (Bethesda, MD).  
20 For making the first strand of cDNA, an oligo dT primer was used. For subsequent amplification from the site of the poly(A) tail, the oligonucleotide provided in the kit or an identical oligonucleotide (ISIS 5586; SEQ ID NO: 107: 5'-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT-3'). For  
25 amplification from the interior of the known sequence, ISIS 6288 was used (SEQ ID NO: 108: 5'-GGGGTAGAATGCGGCGGCAGTATGAAACTCACCAGCG-3'). The DNA resulting from the PCR reaction was gel-purified, digested with Sal I and Bcl I, and then cloned into the Bluescript  
30 plasmid (Stratagene, La Jolla, CA) using standard techniques (Sambrook et al., 1989). The cloned DNA was sequenced using a Sequenase Kit from USB.

The new sequence obtained, from the Bcl I site near the 3' end of the previously known sequence (GenBank  
35 accession number x52479) to the most frequently obtained site of polyadenylation is shown as nucleotides 56-1136 in



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Figure 13. This site is believed to be the 3' end of the short (4kb) PKC $\alpha$  message.

- To extend this sequence and hence obtain sequences specific for the long PKC $\alpha$  message (8.5 kb), the technique of Inverse PCR was performed. Ochman, H., Gerber, A.S. and D.L. Hartl (1988) *Genetics* 120:621-623. This technique was performed three times using a three sets of primers and restriction enzymes. Each round resulted in about 200 bases of new sequence; the total of the new sequence (SEQ ID NO: 104) is shown in bold type (nucleotides 1137-1812) in Figure 13. This sequence is shown extending in the 3' direction beginning at the Bcl I site (TGATCA) near the end of the previously published PKC $\alpha$  cDNA sequence. Finkenzeller et al., *Nucl. Acids Res.* 18:2183 (1990); Genbank accession number X52479. Newly determined sequences begin at nucleotide 56 and are underlined (SEQ ID NO:105). The most common site of polyadenylation, believed to be the 3' end of the short (4 kb) mRNA transcript, is at nucleotide 1136. Sequences downstream from this site, and therefore unique to the long message, are in bold (SEQ ID NO:106).

**Example 18      Antisense oligonucleotides targeted to novel sequences in the 3' UTR of PKC $\alpha$**

- A series of phosphorothioate antisense oligonucleotides, complementary to the novel sequence obtained as described in Example 17, were designed and synthesized. These oligonucleotides were screened on the basis of their ability to cause the reduction or elimination of PKC $\alpha$  RNA in A549 cells 24 hours after the start of treatment. A549 cells were treated with phosphorothioate oligonucleotides at 500 nM for four hours in the presence of the cationic lipids DOTMA/DOPE, washed and allowed to recover for an additional 20 hours. Total RNA was extracted and 20 $\mu$ g of each was resolved on 1.2% gels and transferred to nylon membranes. These blots were probed with a  $^{32}$ P radiolabeled PKC- $\alpha$  cDNA probe and then

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stripped and reprobed with a radiolabeled G3PDH probe to confirm equal RNA loading. The two major PKC- $\alpha$  transcripts (8.5 kb and 4.0 kb) were examined and quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale CA). The 5 oligonucleotides and their activities are shown in Table 13.

Table 13  
Inhibition of PKC $\alpha$  mRNA (both long and short) by  
phosphorothioate antisense oligonucleotides (500 nM)  
Expressed as percent of control mRNA level

10	ISIS#	Sequence	Activity	Target region	SEQ ID NO:
	7416	CAGTGTCCCATGTGCAGGGAG	100%	PKC $\alpha$ long mRNA	109
	7417	AGAACCTGCACAAATAGAGC	100%	PKC $\alpha$ long mRNA	110
15	7418	AGAAACAAGAACCTGCACAA	100%	PKC $\alpha$ long mRNA	111
	7419	GCAAGGGATTTCAGCTAAAC	100%	PKC $\alpha$ long mRNA	112
	7420	AGGGAGGGAAAGCACAGAAG	100%	PKC $\alpha$ long mRNA	113
	7902	AGGGAGGGAAAGCACAGAAG	90%	PKC $\alpha$ long mRNA	113
	7907	TCAGCTCAAAAATAGTCCTC	85%	PKC $\alpha$ long mRNA	114
20	7908	CGAAAGCTGACATGAAGAAA	100%	PKC $\alpha$ long mRNA	115
	7909	GGCGGAGGAACGAGGACGAA	90%	PKC $\alpha$ long mRNA	116
	7911	GCAATGCCACGTGTGTACCA	50%	PKC $\alpha$ long mRNA	117
	7912	TGCAAAACGTATTAATATCC	100%	PKC $\alpha$ short mRNA	118
	7913	TTATAAACATGCAAAATTCA	100%	PKC $\alpha$ short mRNA	119
25	ISIS 7911 (SEQ ID NO: 117) reduced PKC $\alpha$ mRNA levels (both long and short messages) in this preliminary experiment by 50% compared to control. This oligonucleotide is therefore preferred. Further analysis demonstrated that ISIS 7911 selectively reduced the amount of long (8.5 kb) message				
30	during the first six hours of treatment, with a fourfold selectivity at 3 hours post-treatment. By 12 hours after treatment with ISIS 7911, levels of both messages were reduced by over 80%. Time-course data are shown in Figure 14.				

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Nicholas Dean, C. Frank Bennett and Russell T. Boggs
- (ii) TITLE OF INVENTION: Oligonucleotide Modulation of Protein Kinase C
- (iii) NUMBER OF SEQUENCES: 119
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  - (E) COUNTRY: USA
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- (B) COMPUTER: IBM PS/2
- (C) OPERATING SYSTEM: PC-DOS
- (D) SOFTWARE: WORDPERFECT 5.1

## (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: n/a
- (B) FILING DATE: herewith
- (C) CLASSIFICATION:

## (vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 852,852
- (B) FILING DATE: March 16, 1992
- (A) APPLICATION NUMBER: 08/089,996
- (B) FILING DATE: July 9, 1993

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(A) APPLICATION NUMBER: 08/199,779

(B) FILING DATE: February 22, 1994

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## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CCCCAACCAC CTCTTGCTCC 20

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTTCTCGCTG GTGAGTTTCA 20

## (2) INFORMATION FOR SEQ ID NO: 3:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AAAACGTCAG CCATGGTCCC 20

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGATTCACTT CCACTGCGGG 20

## (2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GAGACCCTGA ACAGTTGATC 20

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- 44 -

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CCCGGAAAA CGTCAGCCAT 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTGCCTCAGC GCCCCTTTGC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGTCGCTGCA GTGGCTGGAG 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20

- 45 -

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GCAGAGGCTG GGGACATTGA 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGGCTGGGGA GGTGTTTGTT 20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CACTGCGGGG AGGCTGGGG 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid

- 46 -

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AGCCGTGGCC TTAAATTTT 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATTTTCAGGC CTCCATATGG 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

AAGAGAGAGA CCCTGAACAG 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid



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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GATAATGTTT TGGTTGTAA 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ATGGGGTGCA CAACTGGGG 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GTCAGCCATG GTCCCCCCC 20

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

- 48 -

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CGCCGTGGAG TCGTTGCCCCG 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TCAAATGGAG GCTGCCCGGC 20

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TGGAATCAGA CACAAGCCGT 20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- 49 -

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CATCTTGC GC GGGGAGCC 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TGCGCGCGGG GAGCCGGAGC 20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CGAGAGGTGC CGGCCCGGG 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CTCTCCTCGC CCTCGTCGG 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TGGAGTTTGC ATTACCTAC 20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

AAAGGCCTCT AAGACAAGCT 20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

- 51 -

GCCAGCATGT GCACCGTGAA 20

## (2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

ACACCCCAGG CTCACGATG 20

## (2) INFORMATION FOR SEQ ID NO: 29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CCGAAGCTTA CTCACAATT 20

## (2) INFORMATION FOR SEQ ID NO: 30:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ACTTAGCTCT TGACTTCGGG 20

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## (2) INFORMATION FOR SEQ ID NO: 31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATGCTGCGGA AAATAATTG 20

## (2) INFORMATION FOR SEQ ID NO: 32:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ATTTTATTTT GAGCATGTTC 20

## (2) INFORMATION FOR SEQ ID NO: 33:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TTTGGGGATG AGGGTGAGCA 20

## (2) INFORMATION FOR SEQ ID NO: 34:

- 53 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CCCATCCCCA CAGGCCTGAG 20

## (2) INFORMATION FOR SEQ ID NO: 35:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CGGAGCGCGC CAGGCAGGGA 20

## (2) INFORMATION FOR SEQ ID NO: 36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CCTTTTCCCCA GACCAGCCAT 20

## (2) INFORMATION FOR SEQ ID NO: 37:

## (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:  
GGCCCCAGAA ACGTAGCAGG 20
- (2) INFORMATION FOR SEQ ID NO: 38:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:  
GGATCCTGCC TTTCTTGGGG 20
- (2) INFORMATION FOR SEQ ID NO: 39:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:  
CAGCCATGCC CCCAGAAACG 20
- (2) INFORMATION FOR SEQ ID NO: 40:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20



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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CGACATGCCG GCGCCGCTGC 20

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CAGACGACAT GCCGGCGCCG 20

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GCCTGCTTCG CAGCGGGAGA 20

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

ACAGGTGCAG GAGTCGAGGC 20

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GTCCCGTCTC AGGCCAGCCC 20

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

CCTCACCGAT GCGGACCCTC 20

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

ATTGAACTTC ATGGTGCCAG 20

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

TCTCACTCCC CATAAGGCTA 20

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

TTCCTTTGGG TTCTCGTGCC 20

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

TTCCATCCTT CGACAGAGTT 20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

AGGCTGATGC TGGGAAGGTC 20

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

GTTCTAAGGC TGATGCTGGG 20

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TTCTCGCTGG TGAGTTTC 18

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

TCTCGCTGGT GAGTTTC 17

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

AACTCGAGGT GGCCGCCGTC 20

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

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CGCCTTCGCA TAGCCCTTTG 20

## (2) INFORMATION FOR SEQ ID NO: 56:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GGAAGGGGTG ATTGCGGGCC 20

## (2) INFORMATION FOR SEQ ID NO: 57:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

AACACGCCCA TTGCCACCA 20

## (2) INFORMATION FOR SEQ ID NO: 58:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GTCTCAAGAT GGCCTGCTCG 20

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## (2) INFORMATION FOR SEQ ID NO: 59:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GCGATGGTTC AGCTGGGCCC 20

## (2) INFORMATION FOR SEQ ID NO: 60:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

GCCCTCTCTC TCACTCCCCA 20

## (2) INFORMATION FOR SEQ ID NO: 61:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

CTGGGAAGGT CCGATAGAGG 20

## (2) INFORMATION FOR SEQ ID NO: 62:

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- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:
- AAGGCTGATG CTGGGAAGGT 20
- (2) INFORMATION FOR SEQ ID NO: 63 :
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:
- CGCCGCTCCC TTCCATCTTG 20
- (2) INFORMATION FOR SEQ ID NO:64 :
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:
- CCCCGTAATG CGCCTTGAGG 20
- (2) INFORMATION FOR SEQ ID NO: 65:
- (i) SEQUENCE CHARACTERISTICS:



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- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:  
CTGTCCACCC ACTTGAGGGT 20
- (2) INFORMATION FOR SEQ ID NO: 66:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:  
GCTTCCTCCA TCTTCTGGCT 20
- (2) INFORMATION FOR SEQ ID NO: 67:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:  
CGGTACAGCT TCCTCCATCT 20
- (2) INFORMATION FOR SEQ ID NO: 68:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68

TTGGAAGAGG TGGCCGTTGG 20

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

CCTGTTAAAG CGCTTGCCTT 20

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

TGCAGGTCAG CGGGACGAGG 20

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

GCTCTTGGGA AGGCATGACA 20

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

TTCTTCAACC GCACCAGGAG 20

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

TTCTTCAACC GCACCAGGAG 20

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

CTCTGCCTCT GCATGTGGAA 20

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

TCCTTGACA TGCCGTAGTC 20

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

TCCACGCTGA ACCCGTACTC 20

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

GGAGCGCCCG GCCATCATCT 20

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

GGGCTCGCTG GTGAAGTGTG 20

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

GACGCACGCG GCTCACACC 20

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

GGGTCAATCA CGCGTGTCCTCA 20

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

TCGGAGCCGT GCCCAGCCTG 20

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

CGGGCCAGGT GTGAGGGACT 20

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

CCGCGACGCA GGCACAGCAG 20

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

TGGAAACCGC ATGACAGCCC 20

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

GGTCAGTGCA TCGAGTTCTG 20

(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

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ACTACCATGG TCGGGGCGGG 20

## (2) INFORMATION FOR SEQ ID NO: 87:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

GTCCACCGC ATGGCGCAGC 20

## (2) INFORMATION FOR SEQ ID NO: 88:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

GTTTGCCCGA TGC GCGAGTC 20

## (2) INFORMATION FOR SEQ ID NO: 89:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

TGCAGTTGGC CACGAAGTCG 20



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## (2) INFORMATION FOR SEQ ID NO: 90:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

GTGGGGCATG TTGACGCTGA 20

## (2) INFORMATION FOR SEQ ID NO: 91:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

CCAGAGCAGG GACCCACAGT 20

## (2) INFORMATION FOR SEQ ID NO: 92:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

TCTCCTCGGT TGTCAAATGA 20

## (2) INFORMATION FOR SEQ ID NO: 93:

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- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:
- CGGTGCTCCT CTCCTCGGTT 20
- (2) INFORMATION FOR SEQ ID NO: 94:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:
- AGCCAAAATC CTCCTCTCTG 20
- (2) INFORMATION FOR SEQ ID NO: 95:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:
- CATGAGGGCC GATGTGACCT 20
- (2) INFORMATION FOR SEQ ID NO: 96:
- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 20
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:  
ATCCCTTCC TTGCACATCCC 20
- (2) INFORMATION FOR SEQ ID NO: 97:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:  
CCCCAGGGCC CACCAGTCCA 20
- (2) INFORMATION FOR SEQ ID NO: 98:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (iv) ANTI-SENSE: yes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:  
AGCACCCCCA GGGCCCCACCA 20
- (2) INFORMATION FOR SEQ ID NO: 99:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 20

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

CGTACATCAG CACCCCCAGG 20

(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

CCAGCCATCA TCTCGTACAT 20

(2) INFORMATION FOR SEQ ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

TGCCACACAG CCCAGGCGCA 20

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

TCAGGGCATC AGGTCTTCAC 20

(2) INFORMATION FOR SEQ ID NO: 103:

(i) SEQUENCE CHARACTERISTICS: "

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

CTCTCAGGGC ATCAGGTCTT 20

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1812 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

TGATCAACTG TTCAGGGTCT CTCTCTTACA ACCAAGAACA TTAICTTAGT GGAAGATGGT

60

ACGTCATGCT CAGTGTCAG TTTAATTCTG TAGAAGTTAC GTCTGGCTCT AGGTTAACCC

120

TTCCTAGAAA GCAAGCAGAC TGTTGCCCA TTTGGGTAC AATTGATAT ACTTCCATA

180

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CCCTCCATCT GTGGATTTTT CAGCATTGGA ATCCCCAAC CAGAGATGTT AAAGTGAGCT  
240  
GTCCCAGGAA ACATCTCCAC CCAAGACGTC TTTGGAATCC AAGAACAGGA AGCCAAGAGA  
300  
GTGAGCAGGG AGGGATTGGG GGTGGGGGGA GGCCTCAAAA TACCGACTGC GTCCATTCTC  
360  
TGCTCCATG GAAACAGCCC CTAGAATCTG AAAGGCCGGG ATAAACCTAA TCACTGTTCC  
420  
CAAACTTGA CAAATCCTAA CCCAACCATG GTCCAGCAGT TACCAGTTTA AACAAAAAA  
480  
ACCTCAGATG AGTGTGGGT GAATCTGTCA TCTGTACCC TCCTTGGTTG ATAACGTCT  
540  
TGATACTTTT CATTCTTTGT AAGAGGCCAA ATCGTCTAAG GACGTGCTG AACAGCGTG  
600  
TGAAATCATT TCAGATCAAG GATAAGCCAG TGTGTACATA TGTTCATTTT AATCTCTGGG  
660  
AGATTATTTT TCCATCCAGG GTGCCATCAG TAATCATGCC ACTACTCACC AGTGTGTTC  
720  
GCCAACACCC ACCCCCACAC ACACCAACAT TTTGCTGCCT ACCTTGTTAT CCTTCTCAAG  
780  
AAGCTGAAGT GTACGCCCTC TCCCCTTTTG TGCTTATTTA TTAAATAGGC TGCAGTGTG  
840  
CTTATGAAAG TACGATGTAC AGTAACTTAA TGAAGTGCT GACTCTAGCA TCAGCCTCTA  
900  
CCGATTGATT TTCCCTCCCTT CTCTAGCCCT GGATGTCCAC TTAGGATAA AAAGAATATG  
960  
GTTTTGGTTC CCATTCTAG TTCACGTTGA ATGACAGGCC TGGAGCTGTA GAATCAGGAA  
1020

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ACCCGGATGC CTAACAGCTC AAAGATGTTT TGTTAATAGA AGGATTTTAA TACGTTTTGC  
1080

AAATGCATCA TGCAATGAAT TTTGCATGTT TATAATAAAC CTTAATAACA AGTGAATAGA  
1140

AGGATTTTAA TACGTTTTGC AAATGCATCA TGCAATGAAT TTTGCATGTT TATAATAAAC  
1200

CTTAATAACA AGTGAATCTA TATTATTGAT ATAATCGTAT CAAGTATAAA GAGAGTATTA  
1260

TAATAATTTT ATAAGACACA ATTGTGCTCT ATTTGTGCAG GTTCTTGTTT CTAATCCTCT  
1320

TTTCTAATTA AGTTTTAGCT GAATCCCTTG CTTCGTGCTT TCCCTCCCT GCACATGGGC  
1380

ACTGTATCAG ATAGATTACT TTTTAAATGT AGATAAAAT TCAAAAATGA ATGGCTAGTT  
1440

TACGTGATAG ATTAGGCTCT TACTACATAT GTGTGTGTAT ATATATGTAT TTGATCTAC  
1500

CTGCAAACAA ATTTTATTG GTGAGGACTA TTTTGTAGCT GACACTCCCT CTTAGTTTCT  
1560

TCATGTCACC TTTCGTCTTG GTTCCTCCGC CACTCTTCCT CTTGGGGACA ACAGGAAGTG  
1620

TCTGATCCA GTCTGGCCTA GTACGTTGGT ACACACGTGG CATTGCGCAG CACCTGGGCT  
1680

GACCTTTGTG TGTAGCGTGT GTGTGTGTTT CCTTCTTCCC TTCAGCCTGT GACTGTTGCT  
1740

GACTCCAGGG GTGGGAGGGA TGGGGAGACT CCCCTCTGC TGTGTGTACT GGACACGCAG  
1800

GAAGCATGCT GA  
1812

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(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1757 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

ATGCTACGTC ATGCTCAGTG TCCAGTTTAA TTCTGTAGAA GTTACGCTG GCTCTAGGTT  
60  
AACCCCTTCCT AGAAAGCAAG CAGACTGTTG CCCCATTTTG GGTACAATTT GATATACTTT  
120  
CCATACCCCTC CATCTGTGGA TTTTTCAGCA TTGGAATCCC CCAACCAGAG ATGTTAAAGT  
180  
GAGCTGTCCC AGGAAACATC TCCACCCAAG ACGTCCTTGG AATCCAAGAA CAGGAAGCCA  
240  
AGAGAGTGAG CAGGGAGGGA TTGGGGGTGG GGGGAGGCTT CAAAATACCG ACTGCGTCCA  
300  
TTCTCTGCCT CCATGGAAAC AGCCCCTAGA ATCTGAAAGG CCGGGATAAA CCTAATCACT  
360  
GTTCCCAAAC ATTGACAAAT CCTAACCCAA CCATGGTCCA GCAGTTACCA GTTTAAACAA  
420  
AAAAAACCTC AGATGAGTGT TGGGTGAATC TGTCACTCTGG TACCCTCCTT GGTGATAAC  
480  
TGTCTTGATA CTTTTCATTC TTTGTAAGAG GCCAAATCGT CTAAGGACGT TGCTGAACAA  
540  
GCGTGTGAAA TCATTTCAGA TCAAGGATAA GCCAGTGTGT ACATATGTTT ATTTTAATCT  
600



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CTGGGAGATT ATTTTTCAT CCAGGGTGCC ATCAGTAATC ATGCCACTAC TCACCACTGT  
660  
TGTTGCGCAA CACCCACCCC CACACACACC AACATTTTGC TGCCTACCTT GTTATCCTTC  
720  
TCAAGAAGCT GAAGTGTAGC CCCTCTCCCC TTTGTGCTT ATTTATTAA TAGGCTGCAG  
780  
TGTGCTTAT GAAAGTACGA TGTACAGTAA CTTAATGGAA GTGCTGACTC TAGCATCAGC  
840  
CTCTACCGAT TGATTTTCCT CCCTTCTCTA GCCCTGGATG TCCACTTAGG GATAAAAAGA  
900  
ATATGTTTTT GGTTCCCAT TCTAGTTCAC GTTGAATGAC AGGCCTGGAG CTGTAGAATC  
960  
AGGAAACCCG GATGCCTAAC AGCTCAAAGA TGTTTTGTTA ATAGAAGGAT TTTAATACGT  
1020  
TTTGCAAATG CATCATGCAA TGAATTTTGC ATGTTTATAA TAAACCTTAA TAACAAGTGA  
1080  
ATAGAAGGAT TTTAATACGT TTTGCAAATG CATCATGCAA TGAATTTTGC ATGTTTATAA  
1140  
TAAACCTTAA TAACAAGTGA ATCTATATTA TTGATATAAT CGTATCAAGT ATAAAGAGAG  
1200  
TATTATAATA ATTTTATAAG ACACAATTGT GCTCTATTTG TGCAGGTTCT TGTTCCTAAT  
1260  
CCTCTTTTCT AATTAAGTTT TAGCTGAATC CCTTGCTTCT GTGCTTTCCC TCCCTGCACA  
1320  
TGGGCACTGT ATCAGATAGA TTACTTTTTA AATGTAGATA AAATTTCAAA AATGAATGGC  
1380  
TAGTTTACGT GATAGATTAG GCTCTTACTA CATATGTGTG TGTATATATA TGTATTGAT  
1440

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TCTACCTGCA AACAAATTTT TATTGGTGAG GACTATTTT GAGCTGACAC TCCCTCTTAG  
1500  
TTTCTTCATG TCACCTTTCG TCCTGGTTC TCCGCCACTC TTCCTCTTGG GGACAACAGG  
1560  
AAGTGCTGA TTCCAGTCTG GCCTAGTACG TTGGTACACA CGTGGCATTG CGCAGCACCT  
1620  
GGGCTGACCT TTGTGTGTAG CGTGTGTGTG TGTTCCTTC TTCCTTCAG CCTGTGACTG  
1680  
TTGCTGACTC CAGGGGTGGG AGGGATGGGG AGACTCCCCT CTGCTGTGT GTACTGGACA  
1740  
CGCAGGAAGC ATGCTGA  
1757

## (2) INFORMATION FOR SEQ ID NO:106:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 676 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

TAGAAGGATT TTAATACGTT TTGCAAATGC ATCATGCAAT GAATTTTGCA TGTTTATAAT  
60  
AAACCTTAAT AACAGTGAA TCTATATTAT TGATATAATC GTATCAAGTA TAAAGAGAGT  
120  
ATTATAATAA TTTTATAAGA CACAATTGTG CTCTATTGTG GCAGGTTCTT GTTTCATAATC  
180

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CTCTTTTCTA ATTAAGTTTT AGCTGAATCC CTTGCTTCTG TGCTTTCCTT CCCTGCACAT  
240  
GGGCACTGTA TCAGATAGAT TACTTTTAA ATGTAGATAA AATTTCAAA ATGAATGGCT  
300  
AGTTTACGTG ATAGATTAGG CTCTTACTAC ATATGTGTGT GTATATATAT GTATTTGATT  
360  
CTACCTGCAA ACAAATTTTT ATTGGTGAGG ACTATTTTG AGCTGACACT CCCTCTTAGT  
420  
TTCTTCATGT CACCTTTCGT CCTGGTTCCT CGCCACTCT TCCTCTTGGG GACAACAGGA  
480  
AGTGTCTGAT TCCAGTCTGG CCTAGTACGT TGGTACACAC GTGGCATTGC GCAGCACCTG  
540  
GGCTGACCTT TGTGTGTAGC GTGTGTGTGT GTTTCCTTCT TCCCTTCAGC CTGTGACTGT  
600  
TGCTGACTCC AGGGGTGGGA GGGATGGGA GACTCCCTC TTGCTGTGTG TACTGGACAC  
660  
GCAGGAAGCA TGCTGA  
676

## (2) INFORMATION FOR SEQ ID NO: 107:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: no

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

GGCCACGCGT CGACTAGTAC TTTTTTTTTT TTTTTT 37

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## (2) INFORMATION FOR SEQ ID NO: 108:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

GGGGTAGAAT GCGGCGGCAG TATGAAACTC ACCAGCG 37

## (2) INFORMATION FOR SEQ ID NO: 109:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

CAGTGCCCAT GTGCAGGGAG 20

## (2) INFORMATION FOR SEQ ID NO: 110:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

AGAACCTGCA CAAATAGAGC 20

## (2) INFORMATION FOR SEQ ID NO: 111:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

AGAAACAAGA ACCTGCACAA 20

## (2) INFORMATION FOR SEQ ID NO: 112:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

GCAAGGGATT CAGCTAAAC 20

## (2) INFORMATION FOR SEQ ID NO: 113:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iv) ANTI-SENSE: yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

AGGGAGGGAA AGCACAGAAG 20

## (2) INFORMATION FOR SEQ ID NO: 114:

## (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

TCAGCTCAAA AATAGTCCTC 20

(2) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

CGAAAGGTGA CATGAAGAAA 20

(2) INFORMATION FOR SEQ ID NO: 116:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

GGCGGAGGAA CCAGGACGAA 20

(2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

GCAATGCCAC GTGTGTACCA 20

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

TGCAAAACGT ATTAAATCC 20

(2) INFORMATION FOR SEQ ID NO: 119:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

TTATAACAT GCAAAATTCA 20

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What is claimed is:

1. An oligonucleotide having 5 to 50 nucleotide units specifically hybridizable with a PKC gene or PKC mRNA.
2. The oligonucleotide of claim 1 specifically hybridizable with a translation initiation site, 5' untranslated region, coding region or 3' untranslated region of the PKC gene.
3. The oligonucleotide of claim 1 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.
4. The oligonucleotide of claim 1 wherein at least one of the nucleotides comprises a modification on the 2' position of the sugar.
5. The oligonucleotide of claim 4 wherein the modification is a 2'-O-alkyl or 2'-fluoro modification.
6. The oligonucleotide of claim 4 wherein the modification is a 2'-O-methyl or 2'-O-propyl modification.
7. The oligonucleotide of claim 1 which is a chimeric oligonucleotide.
8. The oligonucleotide of claim 1 wherein said gene or mRNA encodes at least one PKC isozyme.



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9. The oligonucleotide of claim 8 wherein said isozyme is PKC- $\alpha$ , PKC- $\beta$ , PKC- $\gamma$ , PKC- $\eta$ , PKC- $\zeta$  or PKC- $\epsilon$ .
10. The oligonucleotide of claim 9 wherein said gene or mRNA encodes PKC- $\alpha$ .
11. The oligonucleotide of claim 10 comprising SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 52 or 53.
12. The oligonucleotide of claim 9 wherein said gene or mRNA encodes PKC- $\beta$ .
13. The oligonucleotide of claim 12 comprising SEQ ID NO: 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34.
14. The oligonucleotide of claim 9 wherein said gene or mRNA encodes PKC- $\gamma$ .
15. The oligonucleotide of claim 14 comprising SEQ ID NO: 35, 36, 37, 38 or 39.
16. The oligonucleotide of claim 9 wherein said gene or mRNA encodes PKC- $\eta$ .
17. The oligonucleotide of claim 16 comprising SEQ ID NO: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61 or 62.

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18. A pharmaceutical composition comprising the oligonucleotide of claim 1 and a pharmaceutically acceptable carrier or diluent.
19. A chimeric oligonucleotide having SEQ ID NO: 2.
20. The oligonucleotide of claim 19 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.
21. The oligonucleotide of claim 19 wherein at least one of the nucleotide units comprises a modification on the 2' position of the sugar.
22. The oligonucleotide of claim 21 wherein the modification is a 2'-O-alkyl or 2'-fluoro modification.
23. The oligonucleotide of claim 22 wherein the modification is a 2'-O-methyl or 2'-O-propyl modification.
24. A pharmaceutical composition comprising the oligonucleotide of claim 19 and a pharmaceutically acceptable carrier or diluent.
25. A chimeric oligonucleotide having SEQ ID NO: 3.

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26. The oligonucleotide of claim 25 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.

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27. The oligonucleotide of claim 25 wherein at least one of the nucleotides comprises a modification on the 2' position of the sugar.

28. The oligonucleotide of claim 27 wherein the modification is a 2'-O-alkyl or 2'-fluoro modification.

29. The oligonucleotide of claim 28 wherein the modification is a 2'-O-methyl or 2'-O-propyl modification.

30. A pharmaceutical composition comprising the oligonucleotide of claim 25 and a pharmaceutically acceptable carrier or diluent.

31. A chimeric oligonucleotide having SEQ ID NO: 5.

32. The oligonucleotide of claim 31 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.

33. The oligonucleotide of claim 31 wherein at least one of the nucleotide units comprises a modification on the 2' position of the sugar.

34. The oligonucleotide of claim 33 wherein the modification is a 2'-O-alkyl or 2'-fluoro modification.

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35. The oligonucleotide of claim 34 wherein the modification is a 2'-O-methyl or 2'-O-propyl modification.

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36. A pharmaceutical composition comprising the oligonucleotide of claim 31 and a pharmaceutically acceptable carrier or diluent.

37. A method of modulating the expression of PKC in cells comprising contacting the cells with an oligonucleotide having from about 5 to about 50 nucleotide units, said oligonucleotide being specifically hybridizable with a PKC gene or PKC mRNA.

38. The method of claim 37 wherein said oligonucleotide is specifically hybridizable with a translation initiation site, 5' untranslated region, coding region or 3' untranslated region of the PKC gene.

39. The method of claim 37 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate moiety.

40. The method of claim 37 wherein at least one of the nucleotides of the oligonucleotide comprises a modification on the 2' position of the sugar.

41. The method of claim 40 wherein the modification is a 2'-O-alkyl or 2'-fluoro modification.

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42. The method of claim 41 wherein the modification is a  
2'-O-methyl or 2'-O-propyl modification.

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43. The method of claim 37 wherein the oligonucleotide is a chimeric oligonucleotide.

44. The method of claim 37 wherein said gene or mRNA encodes at least one PKC isozyme.

45. The method of claim 44 wherein said gene or mRNA encodes PKC- $\alpha$ , PKC- $\beta$ , PKC- $\gamma$ , PKC- $\eta$ , PKC- $\zeta$  or PKC- $\epsilon$ .

46. The method of claim 45 wherein said gene or mRNA encodes PKC- $\alpha$ .

47. The method of claim 46 wherein said oligonucleotide comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 52 or 53.

48. The method of claim 45 wherein said gene or mRNA encodes PKC- $\beta$ .

49. The method of claim 48 wherein said oligonucleotide comprises SEQ ID: 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34.

50. The method of claim 45 wherein said gene or mRNA encodes PKC- $\gamma$ .



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51. The method of claim 50 wherein said oligonucleotide comprises SEQ ID NO: 35, 36, 37, 38 or 39.

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52. The method of claim 45 wherein said gene or mRNA encodes PKC- $\eta$ .

53. The method of claim 52 wherein said oligonucleotide comprises SEQ ID NO: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61 or 62.

54. A method of modulating the expression of PKC comprising contacting cells with a chimeric oligonucleotide having SEQ ID NO: 2.

55. A method of modulating the expression of PKC comprising contacting cells with a chimeric oligonucleotide having SEQ ID NO: 3.

56. A method of modulating the expression of PKC comprising contacting cells with a chimeric oligonucleotide having SEQ ID NO: 5.

57. A method of detecting in a sample the presence of a PKC gene or PKC mRNA comprising contacting the sample with an oligonucleotide having 5 to 50 nucleotide units specifically hybridizable with said gene or mRNA, and detecting hybridization.

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58. The method of claim 57 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.

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59. The method of claim 57 wherein at least one of the nucleotides of the oligonucleotide comprises a modification on the 2' position of the sugar.
60. The method of claim 57 wherein said gene or mRNA encodes at least one PKC isozyme.
61. The method of claim 60 wherein said gene or mRNA encodes PKC- $\alpha$ , PKC- $\beta$ , PKC- $\gamma$ , PKC- $\eta$ , PKC- $\zeta$  or PKC- $\epsilon$ .
62. The method of claim 61 wherein said gene or mRNA encodes PKC- $\alpha$ .
63. The method of claim 62 wherein said oligonucleotide comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 52 or 53.
64. The method of claim 61 wherein said gene or mRNA encodes PKC- $\beta$ .
65. The method of claim 64 wherein said oligonucleotide comprises SEQ ID NO: 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34.
66. The method of claim 61 wherein said gene or mRNA encodes PKC- $\gamma$ .

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67. The method of claim 66 wherein said oligonucleotide comprises SEQ ID NO: 35, 36, 37, 38 or 39.

68. The method of claim 61 wherein said gene or RNA encodes PKC- $\eta$ .

69. The method of claim 68 wherein said oligonucleotide comprises SEQ ID NO: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61 or 62.

70. A method of treating a condition associated with expression of PKC comprising administering to a mammal or cells thereof with a therapeutically effective amount of an oligonucleotide having 5 to 50 nucleotide units specifically hybridizable with a PKC gene or mRNA.

71. The method of claim 70 wherein said condition is a hyperproliferative disorder.

72. The method of claim 71 wherein said hyperproliferative disorder is psoriasis.

73. The method of claim 71 wherein said hyperproliferative disorder is colorectal cancer.

74. The method of claim 71 wherein said hyperproliferative disorder is lung cancer.

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75. The method of claim 71 wherein said hyperproliferative disorder is breast cancer.

76. The method of claim 71 wherein said hyperproliferative disorder is skin cancer.

77. The method of claim 70 wherein said oligonucleotide is specifically hybridizable with a translation initiation site, 5' untranslated region, coding region or 3' untranslated region of the PKC gene or mRNA.

78. The method of claim 70 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.

79. The method of claim 70 wherein at least one of the nucleotides of the oligonucleotide comprises a modification on the 2' position of the sugar.

80. The method of claim 79 wherein the modification is a 2'-O-alkyl or 2'-fluoro modification.

81. The method of claim 80 wherein the modification is a 2'-O-methyl or 2'-O-propyl modification.

82. The method of claim 70 wherein the oligonucleotide is a chimeric oligonucleotide.

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83. The method of claim 70 wherein said oligonucleotide is in a pharmaceutically acceptable carrier or diluent.

84. The method of claim 83 wherein said carrier or diluent comprises a cationic lipid.

85. The method of claim 70 wherein said gene or mRNA encodes at least one PKC isozyme.

86. The method of claim 85 wherein said gene or mRNA encodes PKC- $\alpha$ , PKC- $\beta$ , PKC- $\gamma$ , PKC- $\eta$ , PKC- $\zeta$  or PKC- $\epsilon$ .

87. The method of claim 86 wherein said gene or mRNA encodes PKC- $\alpha$ .

88. The method of claim 87 wherein said oligonucleotide comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 52 or 53.

89. The method of claim 85 wherein said gene or mRNA encodes PKC- $\beta$ .

90. The method of claim 89 wherein said oligonucleotide comprises SEQ ID NO: 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34.

91. The method of claim 85 wherein said gene or mRNA encodes PKC- $\gamma$ .

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92. The method of claim 91 wherein said oligonucleotide comprises SEQ ID NO: 35, 36, 37, 38 or 39.

93. The method of claim 85 wherein said gene or RNA encodes PKC- $\eta$ .

94. The method of claim 93 wherein said oligonucleotide comprises SEQ ID NO: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61 or 62.

95. A method of treating a condition associated with PKC comprising administering to mammalian cells a therapeutically effective amount of an oligonucleotide having SEQ ID NO: 2, 3 or 5.

96. A method of diagnosing a condition associated with PKC comprising contacting a sample from a mammal suspected of having a condition associated with PKC with an oligonucleotide having 5 to 50 nucleotide units specifically hybridizable with a PKC gene or mRNA, and detecting hybridization.

97. The method of claim 96 wherein said condition is a hyperproliferative disorder.

98. The method of claim 97 wherein said hyperproliferative disorder is psoriasis, colorectal cancer, lung cancer, breast cancer, or skin cancer.



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99. The method of claim 96 wherein at least one of the intersugar linkages between nucleotide units of the oligonucleotide is a phosphorothioate.

100. The method of claim 96 wherein at least one of the nucleotides of the oligonucleotide comprises a modification on the 2' position of the sugar.

101. The method of claim 96 wherein the oligonucleotide is a chimeric oligonucleotide.

102. The method of claim 96 wherein said gene or mRNA encodes at least one PKC isozyme.

103. The method of claim 102 wherein said gene or mRNA encodes PKC- $\alpha$ , PKC- $\beta$ , PKC- $\gamma$ , PKC- $\eta$ , PKC- $\zeta$  or PKC- $\epsilon$ .

104. The method of claim 103 wherein said gene or mRNA encodes PKC- $\alpha$ .

105. The method of claim 104 wherein said oligonucleotide comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 52 or 53.

106. The method of claim 103 wherein said gene or mRNA encodes PKC- $\beta$ .

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107. The method of claim 106 wherein said oligonucleotide comprises SEQ ID NO: 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34.

108. The method of claim 103 wherein said gene or mRNA encodes PKC- $\gamma$ .

109. The method of claim 108 wherein said oligonucleotide comprises SEQ ID NO: 35, 36, 37, 38 or 39.

110. The method of claim 103 wherein said gene or mRNA encodes PKC- $\eta$ .

111. The method of claim 110 wherein said oligonucleotide comprises SEQ ID NO: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61 or 62.

112. The oligonucleotide of claim 9 wherein said isozyme is PKC- $\zeta$ .

113. The oligonucleotide of claim 112 comprising SEQ ID NO: 63, 64, 67, 68, 69, 71, 73, 74, 76, 77, 78, 79, 80, 81, 84 or 85.

114. The oligonucleotide of claim 9 wherein said isozyme is PKC- $\epsilon$ .

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115. The oligonucleotide of claim 114 comprising SEQ ID NO:  
95, 97, 98, 99 or 101.

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116. The method of claim 45 wherein said gene or mRNA encodes PKC- $\zeta$ .

117. The method of claim 116 comprising SEQ ID NO: 63, 64, 67, 68, 69, 71, 73, 74, 76, 77, 78, 79, 80, 81, 84 or 85..

118. The method of claim 45 wherein said gene or mRNA encodes PKC- $\epsilon$ .

119. The method of claim 118 comprising SEQ ID NO: 95, 97, 98, 99 or 101.

120. The method of claim 61 wherein said gene or mRNA encodes PKC- $\zeta$ .

121. The method of claim 120 comprising SEQ ID NO: 63, 64, 67, 68, 69, 71, 73, 74, 76, 77, 78, 79, 80, 81, 84 or 85.

122. The method of claim 61 wherein said gene or mRNA encodes PKC- $\epsilon$ .

123. The method of claim 122 comprising SEQ ID NO: 95, 97, 98, 99 or 101.

124. The method of claim 86 wherein said gene or mRNA encodes PKC- $\zeta$ .

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125. The method of claim 124 comprising SEQ ID NO: 63, 64, 67, 68, 69, 71, 73, 74, 76, 77, 78, 79, 80, 81, 84 or 85.

126. The method of claim 86 wherein said gene or mRNA encodes PKC- $\epsilon$ .

127. The method of claim 126 comprising SEQ ID NO: 95, 97, 98, 99 or 101.

128. The method of claim 103 wherein said gene or mRNA encodes PKC- $\xi$ .

129. The method of claim 128 comprising SEQ ID NO: 63, 64, 67, 68, 69, 71, 73, 74, 76, 77, 78, 79, 80, 81, 84 or 85.

130. The method of claim 103 wherein said gene or mRNA encodes PKC- $\epsilon$ .

131. The method of claim 130 comprising SEQ ID NO: 95, 97, 98, 99 or 101.

132. An isolated nucleic acid molecule comprising a sequence substantially homologous to the sequence set forth in SEQ ID NO: 105.

133. The nucleic acid molecule of claim 132 wherein said nucleic acid molecule is comprised of deoxyribonucleic acid subunits.

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134. The nucleic acid molecule of claim 133 wherein said nucleic acid molecule is double-stranded.

135. An isolated nucleic acid molecule comprising a sequence substantially complementary to the sequence set forth in SEQ ID NO: 105.

136. An antisense oligonucleotide 5 to 50 nucleotides in length comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 105.

137. A pharmaceutical composition comprising the oligonucleotide of claim 136 and a pharmaceutically acceptable carrier.

138. A polynucleotide probe comprising a nucleotide sequence specifically hybridizable with a portion of the nucleic acid molecule of claim 132.

139. A polynucleotide probe comprising a nucleotide sequence specifically hybridizable with a portion of the nucleic acid molecule of claim 135.

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140. An antisense oligonucleotide 5 to 50 nucleotides in length comprising a nucleotide sequence which is specifically hybridizable with the long mRNA transcript of human PKC $\alpha$  and which is not specifically hybridizable with the short mRNA transcript of human PKC $\alpha$ .

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141. The antisense oligonucleotide of claim 140 comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 106.

142. The oligonucleotide of claim 141 comprising the sequence set forth in SEQ ID NO: 115.

143. A pharmaceutical composition comprising an oligonucleotide of claim 139 and a pharmaceutically acceptable carrier.

144. A polynucleotide probe comprising a nucleotide sequence specifically hybridizable to the long mRNA transcript of human PKC $\alpha$ .

145. The polynucleotide probe of claim 144 comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 115.

146. The polynucleotide probe of claim 145 comprising a sequence as set forth in SEQ ID NO: 115.

147. A method for detecting a gene coding for human PKC $\alpha$  in a sample comprising contacting the sample with a polynucleotide probe of claim 138 or claim 139 under conditions which allow for the formation of a polynucleotide duplex between the probe and said gene coding for PKC $\alpha$ ; and



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detecting the presence or absence of a polynucleotide duplex whereby the presence of a polynucleotide duplex indicates the presence of said gene coding for human PKC $\alpha$  in said sample.

148. A method for detecting the long mRNA transcript of human PKC $\alpha$  in a sample comprising contacting the sample with the polynucleotide probe of claim 144 under conditions which allow the formation of a polynucleotide duplex between the probe and the long mRNA transcript of human PKC $\alpha$  and detecting the presence or absence of a polynucleotide duplex whereby the presence of a polynucleotide duplex indicates the presence of said long mRNA transcript of human PKC $\alpha$  in said sample.

149. A method for modulating the expression of PKC $\alpha$  in a cell containing a PKC $\alpha$  gene comprising contacting the cell with an antisense oligonucleotide 5 to 50 nucleotides in length, said antisense oligonucleotide comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 105.

150. A method for specifically modulating the expression of the long mRNA transcript of PKC $\alpha$  in a cell containing a PKC $\alpha$  gene comprising contacting the cell with an antisense oligonucleotide 5 to 50 nucleotides in length, said antisense oligonucleotide comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 106.

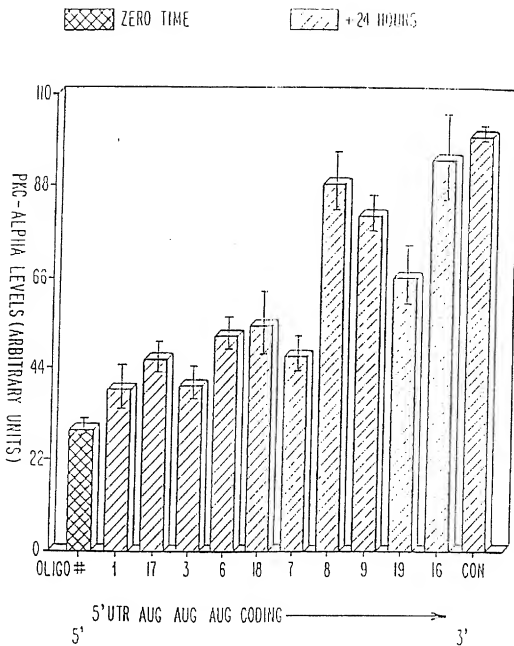
- 104/1 -

151. A method of treating an animal having a condition associated with PKC $\alpha$  comprising contacting said animal with a therapeutically effective amount of an antisense oligonucleotide 5 to 50 nucleotides in length, said antisense oligonucleotide comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 105.

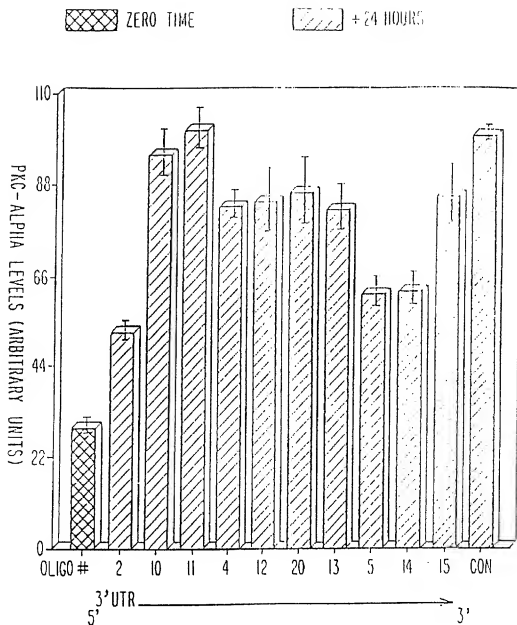
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152. A method of treating an animal having a condition associated with expression of PKC $\alpha$  comprising contacting said animal with a therapeutically effective amount of an antisense oligonucleotide 5 to 50 nucleotides in length, said antisense oligonucleotide comprising a nucleotide sequence specifically hybridizable with a portion of the sequence set forth in SEQ ID NO: 106.

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Fig. 1(a)

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*Fig. 1(b)*

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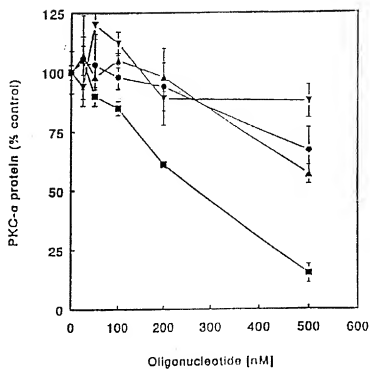


FIG 2

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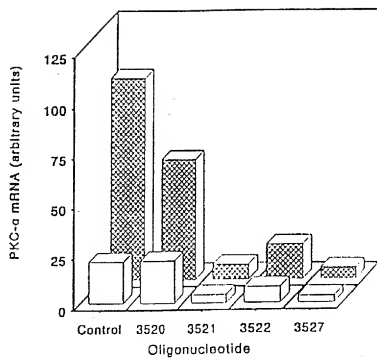


FIG 3

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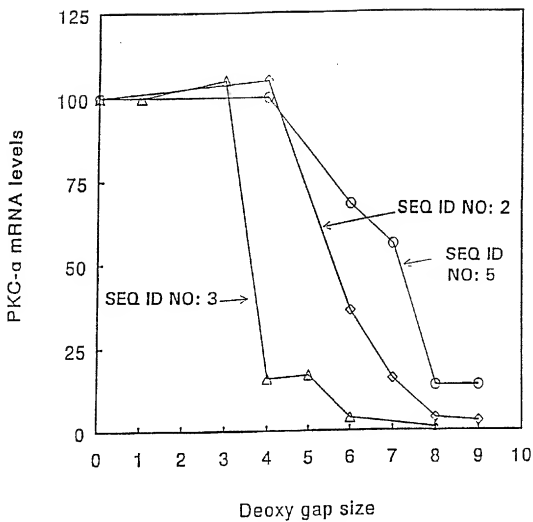


FIG 4



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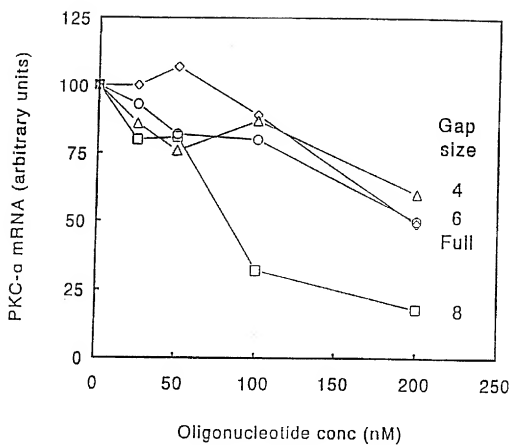


FIG 5

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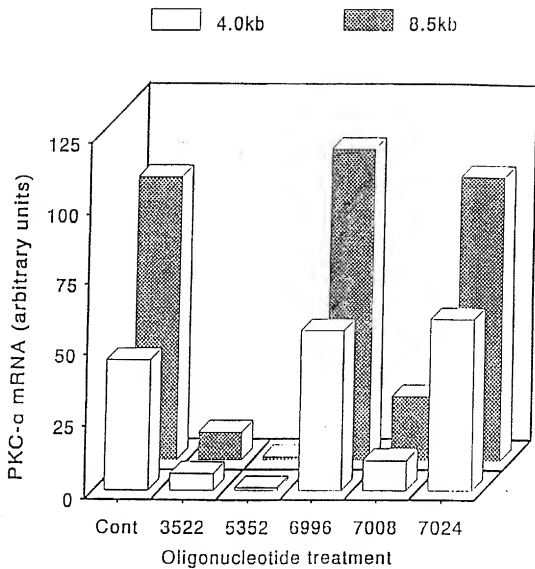


FIG 6

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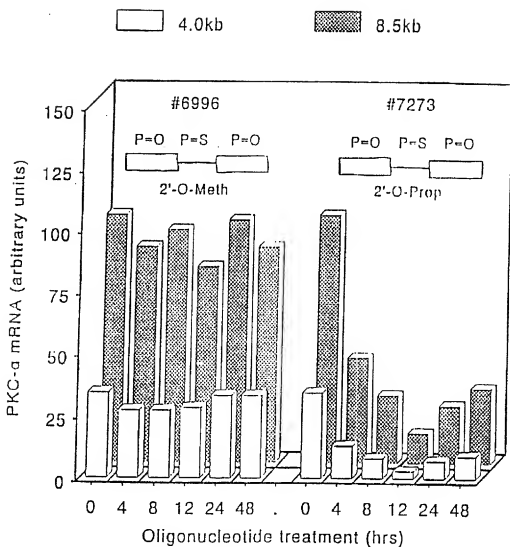


FIG 7

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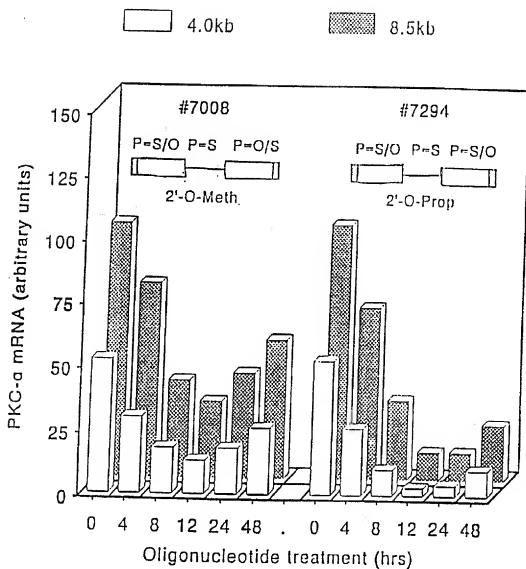


FIG8

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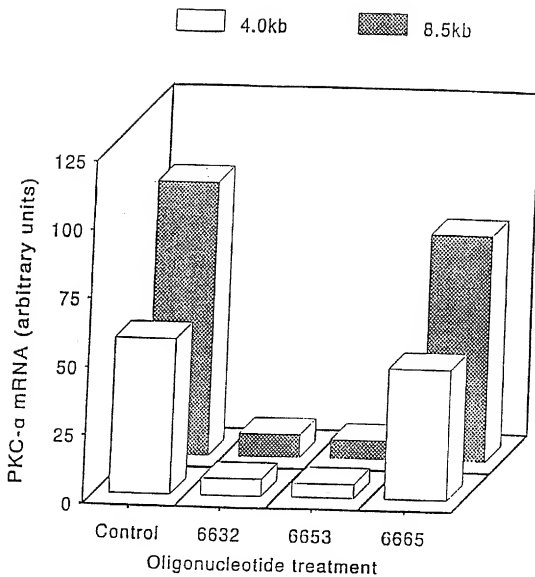


FIG9A

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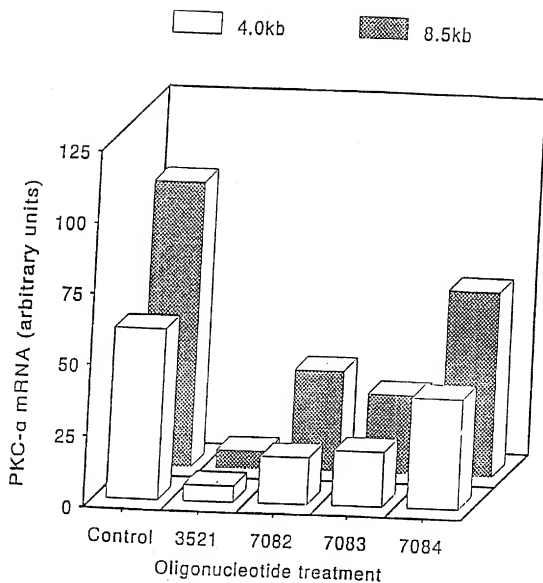
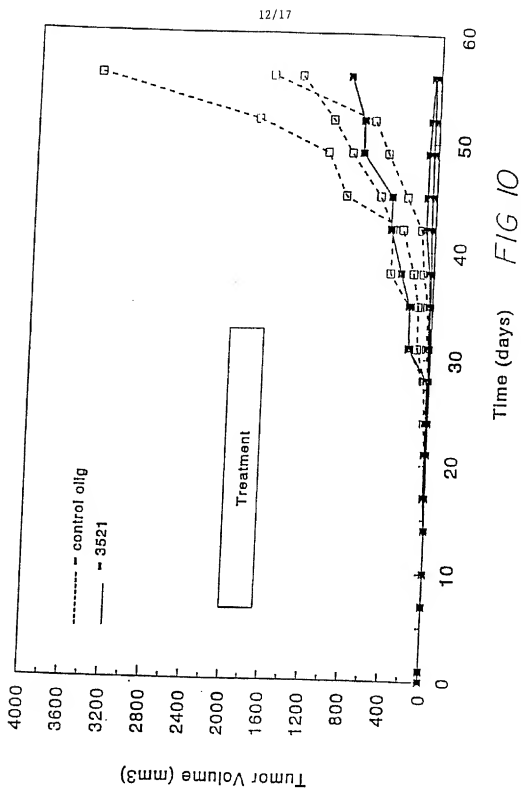


FIG 9B



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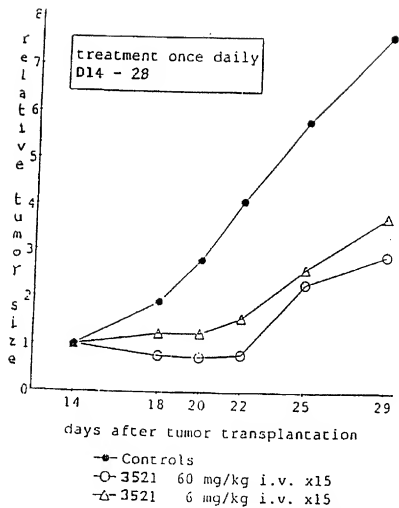


FIG 11A



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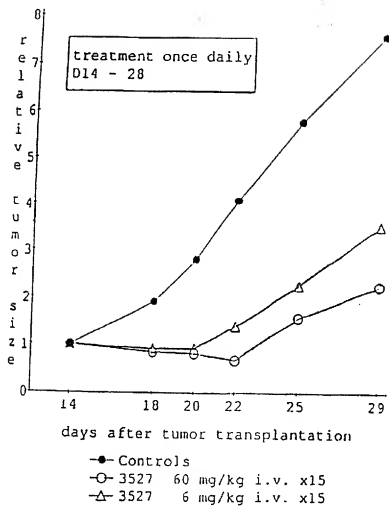
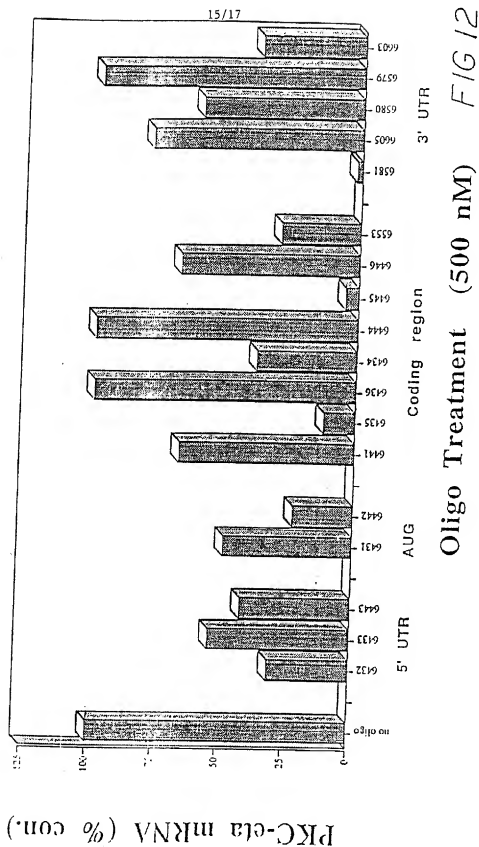


FIG 11B



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FIGURE 13

TGATCAACTG TTCAGGGTCT CTCTCTTACA ACCAAGAACA TTATCTTAT 50  
 GGAAGATGGT ACGTCATGCT CAGTGTCCAG TTTAATCTG TAGAGATAC 100  
 GTCTGGCTCT AGGTTAACCC TTCTTAGAAA GCAAGCAGAC TGTGCCCVA 150  
 TTTTGGGTAC AATTTGATAT ACTTTCCATA CCCCCTATCT GTGGATTTTT 200  
 CAGCATTGGA ATCCCCCAAC CAGAGATGTT AAGTGAAGCT GTCCCAGGAA 250  
 ACATCTCCAC CCAAGACGTC TTTGGAATCC AAGAACAGGA AGCCAAGAGA 300  
 GTGAGCAGGG AGGGATTGGG GGTGGGGGGA GGCCTCAAAA TACCGACTGC 350  
 GTCCATTCTC TCCTCCCATG GAACAGCCCT CTAGAATCTG AAGGCCCGG 400  
 ATAAACCTAA TCCTGTTC CAAACATTGA CAAATCTTAA CCCAACCAT 450  
 GTCCAGCAGT TACCAGTTA AACAAAAAA ACCTCAGATG AGTGTGGGT 500  
 GAATCTGTCA TCTGGTACCC TCCTGGTTG ATAACCTGCT TGATACITTT 550  
 CATCTTTGT AAGAGGCCAA ATCGTCTAAG GACGTTGCTG AACAGCGT 600  
 TGAATTCATT TCAGATCAAG GATAAGCCAG TGTGTACATA GTTTCATTT 650  
 AATCTCTGGG AGTTATTTTT TCCATCCAGG GTGCCATCAG TAATCATGCT 700  
 ACTACTCACC AGTGTGTTT GCCAACACCC ACCCCACAC ACACCAACAT 750  
 TTTGCTGCCCT ACCTTGTTAT CCTCTCAG AAGCTGAAGT GTACGCCCT 800  
 TCCCTTTTG TGCTTATTTA TTTAATAGGC TGCAGTGTGC CTATGAAAG 850  
 TACGATGTAC AGTAACTTAA TGGAAAGTCT GACTCTAGCA TCAGCCCTCA 900  
 CCGATTGATT TTCTCCCTT CTCTAGCCCT GGATGTCCAC TTGGGGATAA 950  
 AAAGAATATG GTTTTGGTTC CCATTCTAG TTTACGTTGA ATGACAGGCC 1000  
 TGGAGCTGTA GAATCAGGAA ACCCGGATGC CTACAGCTC AAGATGTTT 1050  
 TGTAAATAGA AGGATTTTAA TACGTTTTC AAATGCATCA TGCATGAAT 1100  
 TTTGCATGTT TATAATAAAC CTATAAACA AGTGAATAGA AGGATTTTAA 1150  
 TACGTTTTC AAATGCATCA TGCATGAAT TTTGCATGTT TATAATAAAC 1200  
 CTATAAACA AGTGAATCTA TATTATTGAT ATAATCGTAT CAAGTATAAA 1250  
 GAGAGTATTA TAATAATTT ATAAGACACA ATGTGTGCTT ATTTGTGCG 1300  
 GTCTGTGTT CTATCCCTCT TTCTAATTA AGTTTGTGCT GAATCCCTG 1350  
 CTCTGTGCT TTCCCTCCCT GCACATGGGC ACTGTATCAG ATAGATTACT 1400  
 TTTTAAATGT AGATAAAAT TCAAAAATGA ATGGCTAGTT TACGTGATAG 1450  
 ATTAGGCTCT TACTACATAT GTGTGTGAT ATATATGTAT TTGATTCTAC 1500  
 CTGCAACAA ATTTTATTG GTGAGGACTA TTTTGTGCT GACACTCCCT 1550  
 CTATTTTCT TCATGTCACC TTTGCTCCTG GTCTCTCCGC CACTCTTCT 1600  
 CTGCGGACA ACAGGAAGTG TCTGATCCA GTCTGGCCTA GTACGTTGTT 1650  
 ACACACGTGG CATTCGCAG CACCTGGGCT GACCTTTGTT GTAGCGTGT 1700  
 GTGTGTGTTT CCTTCTTCCC TTCAGCCTGT GACTGTGCT GACTCCAGGG 1750  
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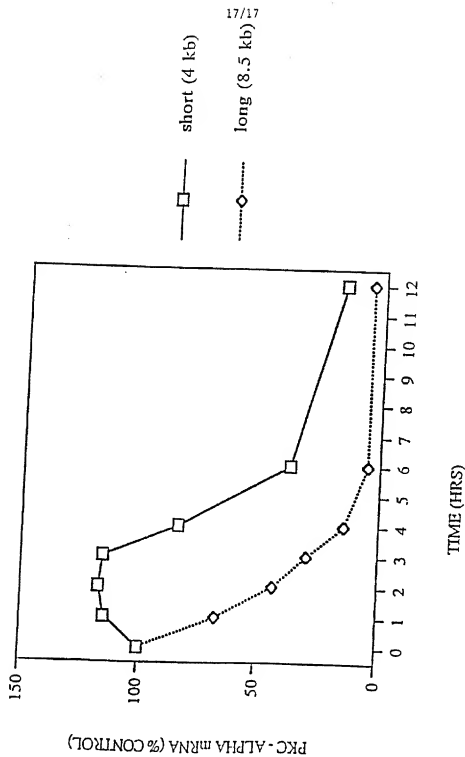


FIGURE 14

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/07770

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C12Q 1/68; A61K 48/00; C07H 21/04

US CL : 435/6; 514/44; 536/24.31, 24.5

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 514/44; 536/24.31, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS

search terms: PKC, antisense, probe, treatment

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Antisense Res. Dev., Volume 1, Number 1, issued 1991,	1-2, 8-10, 12,
---	Farese et al., "Antisense DNA downregulates protein kinase	37-38, 44-46,
Y	C isozymes (beta and alpha) and insulin-stimulated 2-	48
---	deoxyglucose uptake in rat adipocytes", pages 35-42, see	
A	abstract.	3-7, 11, 13-36, 39-43, 47, 49- 56, 132-146, 149-150  70-91, 99-131, 151-152

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* later document published after the international filing date or priority date and not in conflict with the application but cited to understate the principle or theory underlying the invention
* "A" document defining the general state of the art which is not considered to be of particular relevance	* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* "E" earlier document published on or after the international filing date	* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* "G" document member of the same patent family
* "O" document referring to an oral disclosure, use, exhibition or other means	
* "P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

13 OCTOBER 1994

Date of mailing of the international search report

24 OCT 1994

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
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Authorized officer

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Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/07770

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 92-98  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
PARTS OF THESE CLAIMS ARE MISSING FROM PAGE 97
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/07770

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Science, Volume 233, issued 22 August 1986, Coussens et al., "Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways", pages 859-866, see fig. 1 on page 860.	57, 60-61, 64, 66 ----- 58-59, 62-63, 65, 67-69, 147-148
X --- Y	J. Biol. Chem., Volume 268, Number 16, issued 05 June 1993, Godson et al., "Inhibition of expression of protein kinase C alpha by antisense cDNA inhibits phorbol ester-mediated arachidonate release", pages 11946-11950, see second paragraph of Results and Discussion on page 11947.	1-2, 8-10, 37-38, 44-46 ----- 3-7, 11-36, 39- 43, 47-56
X --- Y	Exp. Cell Res., Volume 205, Number 1, issued March 1993, Maier et al., "An oligomer targeted against protein kinase C alpha prevents interleukin-1 alpha induction of cyclooxygenase expression in human endothelial cells", pages 52-58, see page 54.	1-2, 8-10, 37-38, 44-46 ----- 3-7, 11-36, 39- 43, 47-56
X --- Y	Biochemistry, Volume 31, issued 1992, Baxter et al., "PKC-epsilon is involved in granulocyte-macrophage colony-stimulating factor signal transduction: Evidence from microphysiometry and antisense oligonucleotide experiments", pages 10950-10954, see entire document.	1-2, 8-10, 37-38, 44-46 ----- 3-7, 11-36, 39- 43, 47-56